CYTOGENETICS EVOLUTION AND PLANT BREEDING

S. N. PANDEY

CYTOGENETICS EVOLUTION AND PLANT BREEDING

[For University Students of Botany, Zoology & Agriculture]

Surendra Nath Pandey
Plant Physiology Laboratory, Botany Department,
D.A.V. College, Kanpur

S.C.E.R.T.
Library
Calcutta

BHARATI BHAWAN
PUBLISHERS & DISTRIBUTORS

BHARATI BHAWAN (Publishers & Distributors)
Govinda Mitra Road
Patna-800004

Room No. C-11 (First Floor)
College Street Market
Calcutta-700007

S.C.E.R.T, W.B. LIBRARY
Date 30.10.98
Accn. No. 9755

118, Vivekanand Marg
Allahabad-211003

© Author

Published by BHARATI BHAWAN (P & D), Patna-800004.
Printed at Tapan Printing Press, Patna-4.

Rs. 18·00

First Edition, 1979

PREFACE

Recently, science of cytogenetics has grown into a most colourful branch of biology and it has accomodated so much vast discoveries within the last three decades that to synchronise the different chapters on fundamental and classical genetics coupled with modern molecular genetics becomes always unsatisfying to ownself. But researches such as Identification and synthesis of genetic material, Cracking of genetic code, Genetic engineering, Genetic control of growth, differentiation and phenotypic expression, Regulation of gene activities, Biosynthesis of proteins, Laboratory synthesis of life, Revolution in crop breeding and production of new high yielding varieties etc. have proved immensely useful and ensure an excellent human future.

The book, intended to cover the basic concepts of the subject while tracing them to the level of modern researches, would offer an uptodate, lucid and sufficiently illustrated reading to the undergraduate and graduate students of botany, zoology, agriculture and also, to a certain extent, to medical students. Besides, at places it may serve as a source of intellectual stimulation for the subject lovers as it involves facts of our own life and the mysterical machinery of nature.

Here the subject has been so planned as to divide it into several chapters, yet its skeleton part is its organisation into four sub-divisions—The Cell, The Genetics, The Evolution and The Plant Breeding, obviously to include the various Indian Universities syllabi. Chapters on genetics is the nucleus of the book while other ones are like organelles in their importance. These together make it a complete and suitable book.

My experience while dealing with the subject both at graduate and post graduate classes for the last eleven years imposed a feeling to present an uptodate textual matter on the various topics in a way as it could have been explained in the class by the teachers. Though it is a difficult task, yet this attempt may be fruitful in solving many complicated problems of the independent students. At the same time, considering the relative importance of recent discoveries, some sub-chapters have been raised to separate chapters to invite special attention of readers.

I would be remiss if I fail to express my sincere thanks to Mr. Arif Ali for his help in drawings; to Dr. W. M. Laetsch, U.S.A.; Dr. Ruth Sagar, U. S. A.; Dr. E. Weier, U.S.A.; Dr. Keith Porter, U.S.A.; Dr. D. W. Fawcett, U.S.A.; and to Dr. Sultana Rizvi, N.B.G., Lucknow, India for permitting certain photographs to use in the book. Lastly, it needs no apology to record here my indebtedness to my eximious bosom friend whose untiring feeling ceaselessly encouraged me to complete this work.

Suggestions and criticisms for the betterment of the book would be most welcomed and I shall feel obliged for the same.

Surendra Nath Pandey

CONTENTS

Chapter 1. THE CELL—AN INTRODUCTION

Introduction; Cell size; Cell shape; Cell number; Organisation of cell, The protoplasm—physical porperties and chemical properties; Vacuoles; Procaryotic cell; Animal and Plant cells. ... 1

Chapter 2. CELL WALL AND BIOMEMBRANES

CELL WALL—Middle lamella, Primary wall, Secondary wall, Organisation of cell wall, Function of cell wall; BIOMEMBRANES—Composition of membranes—membrane protein, enzymes, carrier proteins; membrane lipids; membrane polysaccharides and glycolipids; membrane RNA; membrane DNA; Some other components; Models of biomembrane structure—(i) Danielli—Davson model, (ii) Unit membrane hypothesis, (iii) Kavanau's lipid pillars, (iv) Hydrophobic binding models, (v) Benson's model, (vi) Lenard and Singer's model, (vii) Mosaic membrane concept, (viii) Composite model, (ix) Fluid mosaic model; Some special structures of biomembranes—Microvilli, Desmosomes; Functions of biomembranes; Phagocytosis, Pinocytosis; General questions. ... 10

Chapter 3. SOME CYTOPLASMIC ORGANELLES

PLASTIDS—Chromoplasts, Leucoplasts, Chloroplasts; *Chloroplast*—introduction, structure, ultrastructure—Branton and Park model, Weier and Benson model; the photosynthetic units; chemical composition; Function; Origin of chloroplast; *Mitochondria*—structure, function, origin of mitochondria; *Endoplasmic reticulum*; *Golgi Complex*—ultra structure and function; *Ribosomes*; *Lysosomes*—structure, functions, origin; *Transosomes*; *Glyoxysomes*; *Microtubules*; *Peroxisomes*, *Spherosomes*; *Centrosomes*; *Cilia and flagella*, General questions. ... 25

Chapter 4. NUCLEUS AND THE CHROMOSOMES

THE NUCLEUS—Hammerling's experiment, structure and composition of nucleus; THE CHROMOSOMES—Morphology of Chromosomes, structure of chromosomes, chemistry of chromosomes, fine structure of chromosomes; SOME SPECIAL TYPES OF CHROMOSOMES—Salivary gland chromosomes, Lamp-brush chromosomes, Supernumery chromosomes, Sex-chromosomes, General questions. ... 48

Chapter 5. THE CELL CYCLES AND CELL DIVISIONS

THE CELL CYCLES; CELL DIVISIONS—Amitosis, Mitosis, Meiosis; Comparison between mitosis and meiosis; Physiology of cell divisions; General questions. ... 63

Chapter 6. LINKAGE AND CROSSING OVER

LINKAGE—Linkage in *Drosophila*; linkage in *Zea mays*; Classification of linkage; Theories on Linkage—Differential multiplication theory, chromosome theory of linkage; Importance of linkage; CROSSING OVER—Mechanism of crossing over—The classical hypothesis, Janssens' hypothesis, Torsion theory, Belling's hypothesis; Chromosomal maps; Cytological basis of crossing over; Stern's experiment in *Drosophila*; Significance of crossing over; General questions. ... 90

Chapter 7. CYTOLOGY IN RELATION TO TAXONOMY

Introduction, Chromosome number, Chromosome morphology, Chromosome behaviour in crosses, Concluding remarks; General questions. ... 110

Chapter 8. MENDEL'S LAWS OF INHERITANCE

MENDELISM—Life of Mendel, Pre-Mendelian era, G. J. Mendel, Mendel's experiments, Terminology, Mendel's laws of Inheritance —Law of dominance, Law of segregation—mechanism of segregation, Law of independent assortment; Back cross and Test cross; Trihybrid and polyhybrid crosses, Problems On Mendelism. ... 115

Chapter 9. MODIFICATIONS OF MENDEL'S LAWS

Incomplete dominance, Interaction between dominant factors, Complementary factors, Supplementary factors, Epistasis, Inhibitory factors, Duplicate factors, Polymerism, Lethal factors, Pleiotropy, Multiple factors, General questions. ... 142

Chapter 10. CHROMOSOMAL ABERRATIONS

Introduction, Deficiencies, Duplications, Translocations, Inversions, General questions. ... 156

Chapter 11. EXTRA-NUCLEAR INHERITANCE

Introduction; Cytoplasmic genetic system; Role of genes in cytoplasmic inheritance; Plastid inheritance—iojap genes in *Zea mays*, Plastid development in *Oenothera*, Leaf variegation in *Mirabilis jalapa*; Other examples of cytoplasmic inheritance :—(i) Influence of cytoplasm on genome in *Epilobium*, (ii) Killer characters in *Paramecium*, (iii) Petite colonies in yeast, (iv) Poky characters in *Neurospora*, (v) Inheritance of CO_2 sensitivity in *Drosophila*, (vi) Streptomycin resistance in *Chlamydomonas*, (vii) Cytoplasmic male sterility, (viii) Inheritance of dextral and sinistral coiling and (ix) Mouse 'milk factor'; General questions. ... 165

Chapter 12. POLYPLOIDY

Introduction; Classification and terminology; Euploidy—Haploids, Autopolyploidy, Autotriploids, Autotetraploids; Allopolyploidy, Genomic allopolyploids, Segmental allopolyploids, Autoallopolyploids; Aneupolyploidy (heteropolyploidy), Trisomics, Secondary trisomics, Monosomic aneuploidy, Nullisomic aneupolidy; Induction of polyploidy; Polyploidy in animals; Polyploidy in evolution; Polyploidy and agriculture; General questions. ... 180

Chapter 13. SEX DETERMINATION AND SEX REVERSALS

Introduction ; Brief history ; Chromosomal theory of sex determination, Metabolic theory of sex determination, Nutritional theory of sex determination, Quantitative theory of sex determination, Genic balance theory of sex determination ; Hormonal control of sex determination; Some examples of sex determination—*Drosophila*, Man, *Melandrium*, *Zea mays*, *Bryonia* and *Rumex* ; Gynandromorphs, Kinds of gynanders, Origin of gynanders; Sex-reversals, causes of sex reversals; General questions. ... 201

Chapter 14. SEX-LINKED INHERITANCE

Sex-linked inheritance in *Drosophila* ; Sex-linked inheritance in man—colour blindness, Hemophilia; Sex-linked genes in poultry; Sex-linkedlethals in *Drosophila*; Non-disjunction of Sex chromosomes in *Drosophila* ; General questions. ... 218

Chapter 15. MUTATIONS

Introduction; Detection of mutation—The ClB method, Muller's 5 method, Detection of mutation in plants; Classification of mutation; Mechanism of mutation—spontaneous mutation; Induced mutation : Radiations, Chemical mutagens—Chemically reacting compounds, Substitution of base analogues, Mutation by misincorporation, transition, transversion; Mutations and crop improvement ; General questions. ... 230

Chapter 16. GENETICS OF NEUROSPORA

Introduction, Life cycle, Genetic applications of *Neurospora*—Inheritance test in *Neurospora*, Detection of biochemical mutations, Genetic control of the biosynthesis of argenine, methionine and tryptophan. ... 247

Chapter 17. THE GENE

The relation of genes and chromosomes, Defining genes, Nature of genes, Size of genes, Gene is the ultimate unit of recombination, Gene is the ultimate unit of function—*Recon*, *Muton*, *Cistron*, *Complon*, *Replicon*, *Transcripton*, *Codon*, *Operon*; Stability of genes, Position effect—Position effect in *Drosophila*, Ac Ds position in maize;

Gene action, Physical studies and chemical composition, Gene at the core of crime. ... 254

Chapter 18. ONE GENE—ONE ENZYME HYPOTHESIS

Introduction, Alcaptonuria, Floral pigmentation, Eye pigment in *Drosophila*, Tryptophan-less mutants of *Neurospora*, Sickle-cell anaemia, Conclusion, Few complications concerning hypothesis—Pleiotropy, Suppression, Controlling genetic units; Discussion. ... 262

Chapter 19. EVIDENCES : DNA AS A GENETIC MATERIAL

Introduction, Bacterial transformations—transformation in *D. pneumoniae*, mechanism of transformation, transformation in *B. subtilis*, transformation in streptomycin resistant bacteria; Transformation in blue green algae; Bacteriophage infection; Transduction; Infective RNA, General Questions. ... 269

Chapter 20. THE NUCLEIC ACIDS [DNA & RNA]

DNA—DNA content of nuclei, components of DNA, structure of DNA—basic structure of DNA, model of DNA, single stranded DNA, Replication of DNA, Meselson and Stahl's experiment, Cairn' autoradiography experiment; Rate of replication; RNA—general, Ribosomal RNA messenger RNA and transfer RNA. General questions. ... 278

Chapter 21. BIOSYNTHESIS OF PROTEINS

General, Formation of *m* RNA, Polysome, *t*RNA, Amino acid activation, Translation, Composite behaviour of ribosomes, Formation of polypeptide chain, General questions. ... 306

Chapter 22. THE GENETIC CODE

Genetic code—*m* RNA genetic code dictionary, Nature of genetic code, General questions. ... 316

Chapter 23. REGULATION OF GENE ACTIVITY

General, Regulatory activities in cytoplasm, Regulatory activities in the nucleus, Operon model—Inducible systems, Repressible system, General questions. ... 321

Chapter 24. ORIGIN OF LIFE

Introduction ; Chemical evolution; Pre-biotic Chemical evolution, amino acids, carbohydrates, purines and pyrimidines, nucleosides and nucleotides, porphyrins, proteins; Origin of first living system, Coacervate drops, proteinoid microspheres; Artificial synthesis of life. ... 326

Chapter 25. EVIDENCES OF EVOLUTION

Introduction; Evidences from embryology, Ontogeny of hearts, Ontogeny of aortic arches; Evidences from comparative anatomy; Evidences from vestigeal organs; Evidences from physiology; Evidences from genetics; Evidences from domestication; Evidences from classification; Evidences from geographical distribution; Evidences from fossils, *Archaeopteryx*, evolution of horse. ... 334

Chapter 26. DIFFERENT THEORIES ON EVOLUTION

The theory of Lamarck—Lamarck, Lamarckism, Demerits of Lamarckism, ,Objections; The theory of Darwin, Darwin, Darwinism, Origin of new species, Evidences in favour of Darwinism, Experimental evidences, Objections to Darwinism; Neo-Darwinsim; Weismann's theory of germ plasm; Mutation theory of de Vries—differences between variations and mutations, advantages of mutation theory, limitations of mutations; Synthetic theory of evolution. ... 347

Chapter 27. PLANT BREEDING

Introduction, Aims and objectives, Methods of Crop improvement, Selection, Mass selection—application and uses, advantages, limitations, achievements, Pure line selection, clone and clonal selection, Hybridisation—different types, procedures, harvesting and raising of F_1 generation, hybridisation methods, difficulties and precautions, achievements. ... 360

Chapter 28. BREEDING FOR DISEASE RESISTANCE

Introduction, Application of resistant breeding, Genetics of breeding resistance, Methods, Achievements. ... 373

Chapter 29. THE HETEROSIS OR HYBRID VIGOUR

Introduction, Theories on causes of heterosis, Utilisation of hybrid vigour in crop improvement, Hybrid corn, Hybrid pearl millet. ... 379

Chapter 30. CROP BREEDING IN INDIA

Introduction, Wheat, Rice, Potato, Sugar cane, Cotton. ... 386

Appendix X. : SCIENTISTS' CONTRIBUTION ... 398

SELECTED READINGS ... 401

INDEX ... 406

CHAPTER 1

THE CELL: AN INTRODUCTION

Cell is the most enigmatic mystery of nature about which it is believed that the day scienitsts will understand it fully, they would be able to unravel the secret of life and death. In general, life is a dynamic equilibrium of hundreds and thousands and millions of chemical and biochemical reactions going on in between non-living substances at ionic and molecular levels and a cell is its unit of structure, function and heredity. The word "cell" was coined by Robert Hooke (1665) when with the help of compound microscope, he observed the texture of cork's thin section as composed of small spaces surrounded by firm cell wall. The whole structure looked like honey-comb. He was thrilled to observe such structure and called them cells. Cell is a latin word derived from *cellula* which means small apartments.

With the advent of time more advanced types of microscopes became available which helped Robert Brown (1831) to find a spherical body in each cell. He called it nucleus. By this time many scientists flung in this field. Mirbel (1908-09) suggested that "*plants are formed by a membranous cellular tissue*". While Lamarck (1819) advanced this statement by saying "*no body can have life, if its constituent parts are not cellular tissue or are not formed by cell,*" M. J. Schleiden and Theodor Schwan (1839) propounded the **cell theory** stating "*The cells are organisms; and animals as well as plants are aggregates of these organisms, arranged in accordance with definite laws*" and in 1855, Virchow concluded that "*Omnis cellula-e-cellula*", i.e. new living cells arise only by reproduction of pre-existing living cells. Almost at the same time Von Mohl (1846) observed a viscous, jelly-like mass surrounding each nucleus and then enunciated a concept that "*protoplasm is the physical basis of our life*". Max Schultze (1861) established the similarity between the protoplasm of animal and that of plant cells. Later it was called **protoplasm theory** by O. Hertwig (1892). The cells of all the living organisms though varying to a great extent in size, mass, shape, structure and function have many things in common.

The cell has been variously defined as "*Cell is the structural and functional unit of plants and animals*" or "*A cell is a small mass of living matter containing a nucleus*", or "*Cell is the unit of life*" or "*Cell is a mass of protoplasm surrounded by a thin membrane*".

However, there are many exceptions to cell theory, e.g. virus. Viruses do not fit in the definition of cell and they are therefore often described as **living chemicals**. They are supposed to be primitive organisms that fail to reach a cellular state. Among other examples, *Vaucheria* (an alga) and *Rhizopus* (a fungus) may also be considered. Their living substances are enclosed in a wall and there is hardly any organisation like that of a cell. It is therefore difficult to accomodate these kinds of units in the context of cell theory.

Cell Size

The size of cells varies widely. The smallest living cells are bacteria (0·2-5·0 μ) which are just at the limits of resolution of the light microscope. The largest cell is the *ostrich* egg which measures about six inches. The largest *unicellular* plant is *Acetabularia*. Thus, the ratio of linear dimensions between the largest and smallest cell is about 75000 : 1 and the ratio of their volume $75{,}000^3$: 1. A vast majority of cells ranges in between 0·5 to 20 μ in dia. The flesh cells of *Citrus* and *Cucurbita* are large enough to be seen by naked eyes. However, the following table reveals some interesting average measurements satisfying the curious eyes.

Name of the cell	*Measurement*	*Name*	*Measurement*
Ostrich egg	170×135 mm.	Typhoid bacillus	2·4×0·5 micron
Hen egg	60×45 mm.	Influenza bacillus	0·5×0·2 ,,
Humming bird egg	13×8 mm.		
Human egg	0·1 mm.	Pneumococcus bacterium	500×200 m μ
Amoeba	100 microns (μ)	Bacterial virus	80 m μ
Sea urchin egg	70 ,,		
Liver cell	20 ,,	Protein molecule	10 m μ
Red blood cells	7 ,,	Haemoglobin molecule	7 m μ

Cell Shape

The shape of cells also varies depending upon their function, needs of the organisms and environmental conditions. The fact can be easily substantiated when the various shapes of unicellular organisms such as *Amoeba*, *Paramoecium*, *Chlamydomonas*, Yeast, Bacteria etc. are visualised. Some bacteria are rods, spirals and even comma-like while *Acetabularia* consists of a stalk and a cap upto 10 cm. in height. The shape of the cell in multicellular organisms shows some regularity where it is partly controlled by the mutual pressure of the cells. However, in these cases, some specialised cells modified for various functions show typical shapes, e.g. cells of glandular hairs of leaf, guard cells and root hairs.

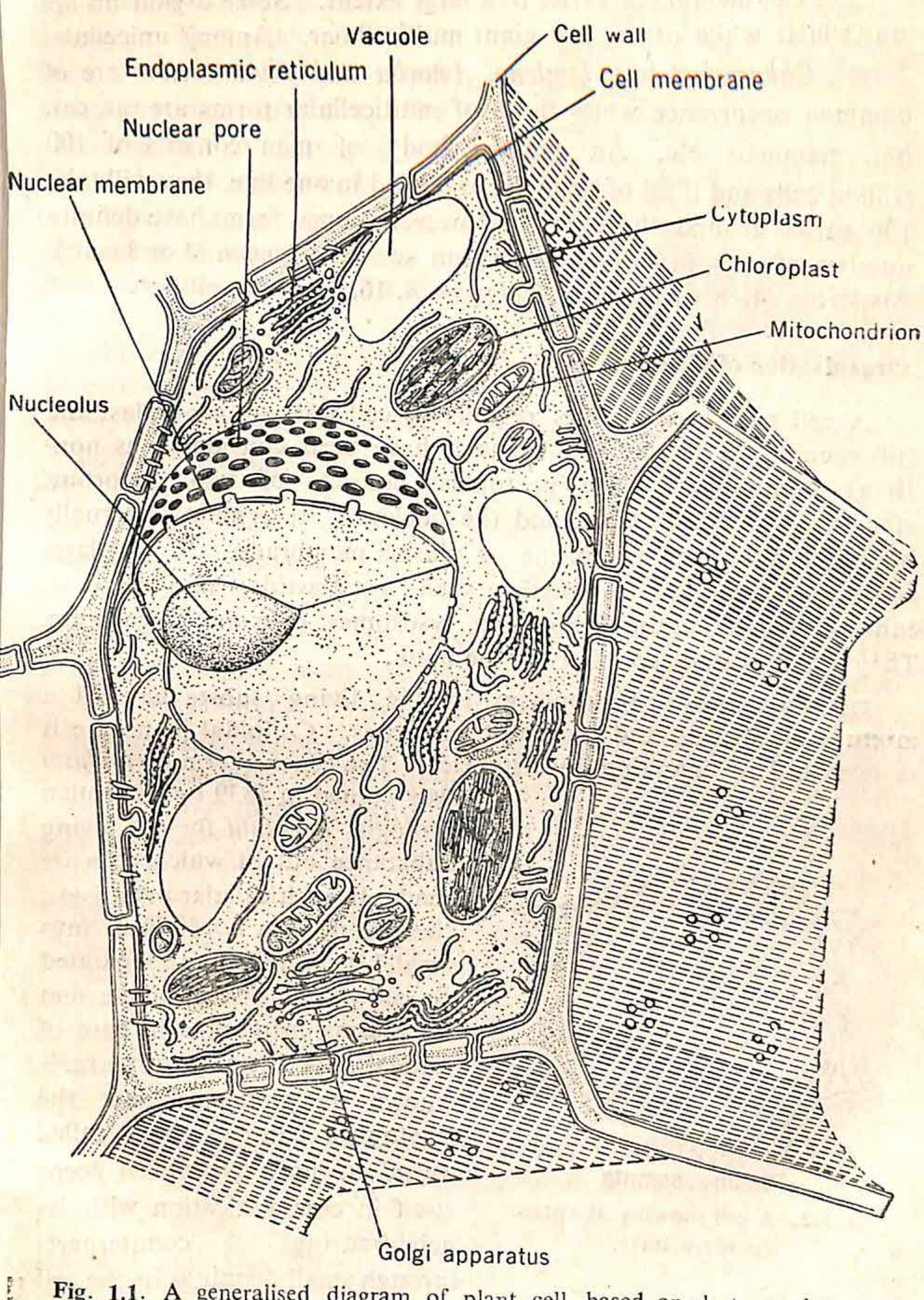


Fig. 1.1. A generalised diagram of plant cell based on electron microscopic studies. The perforations on the wall indicate the regions of plasmodesmata.

Cell Number

The cell membrane varies to a large extent. Some organisms are unicellular while others are giant multicellular. Among unicellular forms, *Chlamydomonas*, *Euglena*, *Amoeba* and *Paramoecium* are of common occurrence while those of multicellular forms are rat, cat, bat, mammals etc. An average body of man consists of 100 trillion cells and if all of them are extended in one line, they will take 100 turns around the earth. However, some forms have definite number of cells in their organisation such as *Gonium* (4 or 8 cells), *Pandorina* (4, 8 or 16 cells), *Eudorina* (8, 16, 32 or 64 cells) etc.

Organisation of Cell

A cell has three distinct regions (i) cell wall, (ii) protoplast and (iii) vacuole. Cell wall and vacuoles have been considered as non-living components. The protoplast consists of two important structures—the cytoplasm and the nucleus. It remains externally bounded by a cell membrane or plasma membrane. A cytoplasm contains several cell organelles such as plastids, mitochondria, endoplasmic reticulum, ribosomes, lysosomes, cilia and flagella etc. They are discussed separately in the book.

THE PROTOPLASM : Protoplasm is the living substance and a mixture of organic and inorganic materials. Colloidal in nature it is semifluid of changing consistencies. The general term *protoplasm* was coined in 1839 by Bohemian biologist *Purkinja* for the living substance out of which cells are made. In multicellular organisms, the protoplasm is divided into small bits bounded and separated by cell wall in case of plants and by plasma membrane in case of animals. A bit of protoplasm is known as *protoplast* and the protoplast community is called *symplast*. Each protoplast keeps itself in communication with its neighbouring counterparts through small openings in the cell boundary known as plasmodesmata.

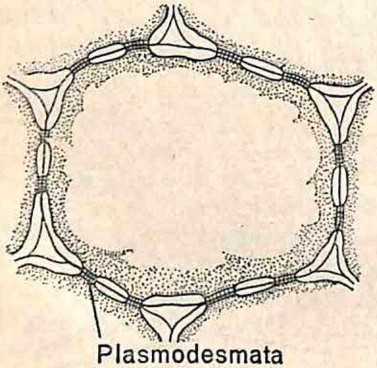


Fig. 1.2. A cell showing the plasmodesmata

Physical properties : The protoplast appears as a colourless, elastic, colloidal semifluid containing numerous tiny granules and droplets. The tiny particles show erratic and random movements within protoplast. The phenomenon is referred as "Brownian movement". In plant cells, vacuoles are also present and there protoplast shows a state of movement around these vacuoles. It is referred as cyclosis which can easily be seen in the cells of *Hydrilla*, *Eleocharis*, *Chara*, *Nitella*, *Tradescantia* etc.

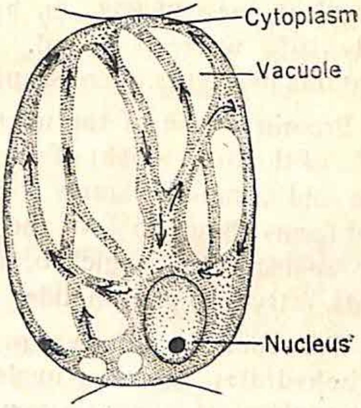


Fig. 1.3. A cell showing cyclosis.

Because of the presence of both living and non-living cell inclusions, the protoplast forms a "*complex polyphase colloidal system*". It also possesses sol-gel properties and frequently undergoes such transformations by taking in or losing water. Optically the protoplast is transparent and permits to pass through the wave length of visible spectrum. The tyndall effect is also observed. The viscosity of protoplast is affected by mechanical injury, electric shocks, temperature and pH changes.

Various theories have been proposed to establish the physical nature of protoplast. Summarising these, a protoplast is reticular as referred by some scientists, while others called it fibrillar, granular or alveolar, respectively.

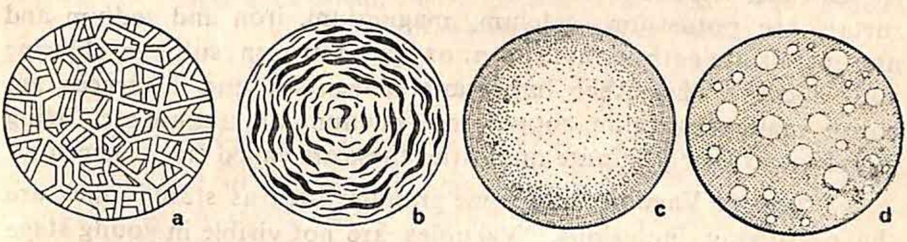


Fig. 1.4. Various theories on the physical nature of protoplast, a—reticular, b—fibrillar, c—granular and d—alveolar.

To the best, the protoplasm is considered now-a-days as a complex polyphasic colloidal system.

Chemical nature : Water is the chief constituent of an active protoplast and normally constitutes 90% of the system. In woody parts of the plants, water is about 50% but in soft parts 75%; in succulent parts 85-95%, in hydrophytes 95-98% and in dry seeds only 10% water is found. The remaining part of the protoplast contains two types of constituents, i.e. organic and inorganic.

Protein is one of the main constituents which constitutes about 70% of the total weight of protoplasm. Carbohydrates exist in both free and combined states with protein in the form of nucleoprotein and forms about 1·5% of the total protoplasm. About 2% of the protoplasm is lipid which occurs in the form of natural fats, fatty acids, fatty oils, phosphatides, waxes and steroides.

Thus common prevalent organic compounds of a protoplasm are carbohydrates, proteins, nucleic acids, lipids, fats, phosphatides etc. The analyses of inorganic components of the protoplasm have shown the presence of about 40 elements. Some of them are found in extremely minute quantities. The average chemical composition of plant body may be as follows.

Elements	% *wt.*	*Elements*	% *wt.*
Oxygen	62	Hydrogen	10
Carbon	20	Nitrogen	3
Phosphorous	1·4	Sulphur	0·14
Chlorine	0·16	Potassium	0·11
Iodine	0·014	Magnesium	0·07
Calcium	2·50	Iron	0·01
Other trace elements	0·756	Sodium	0·1

Thirteen elements are constantly found in plant ash. Among metals are potassium, calcium, magnesium, iron and sodium and non-metals are carbon, hydrogen, oxygen, nitrogen, sulphur, chlorine and silicon. The other important elements found in traces are boron, manganese, zinc, copper, molybdenum, aluminium etc. Being found in extremely minute quantities these are called trace elements.

VACUOLES : Vacuoles and some granules such as starch ones are the non-living inclusions. Vacuoles are not visible in young stage but as the cell grows in size and enlarges, they begin to make their appearance like small droplets which go on coalescing with each other to form a bigger vacuole at maturity. At maturity the size of vacuole may enlarge to such an extent that cytoplasm is left only in the form of thin peripheral cytoplasmic layer enclosing the nucleus. Surrounded by the tonoplast, a vacuole contains cell sap with atmospheric gases, minerals, sugars, organic acids (such as acetic acid, malic acid and tartaric acid) and at times anthocyanin pigments. The various colours of petals are due to the presence of different anthocyanin pigments present in vacuoles. Some solid bodies of aleurone grains and soluble proteins are also present in it.

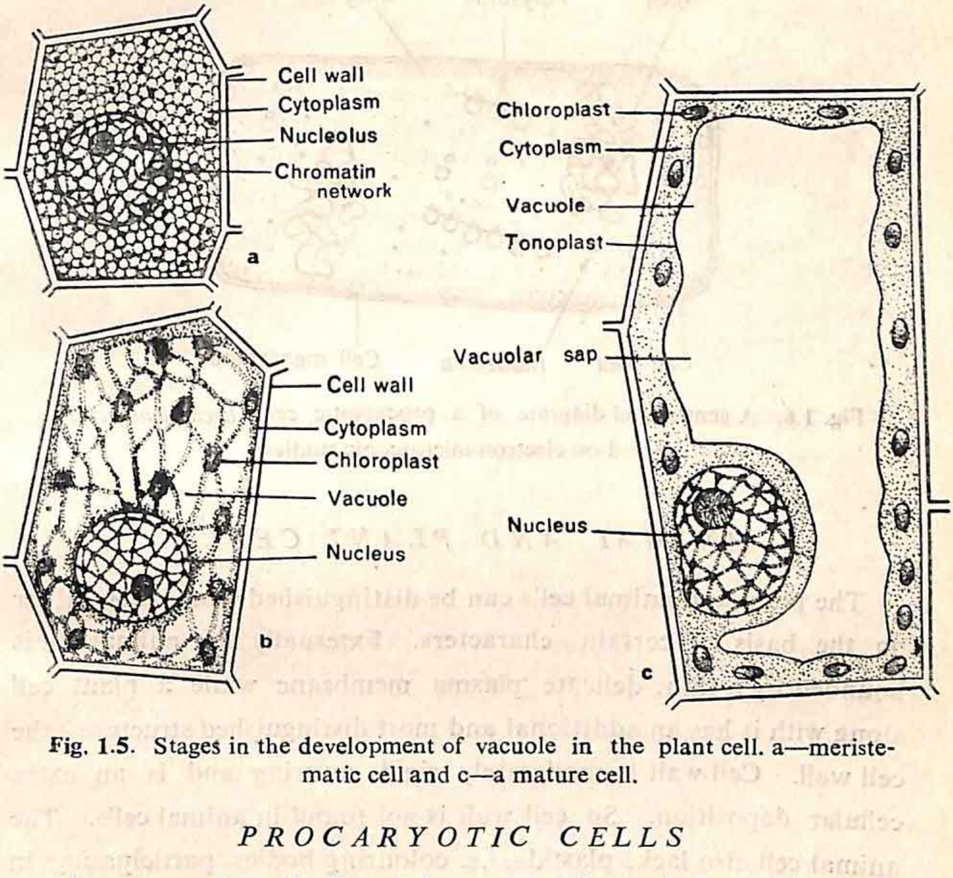


Fig. 1.5. Stages in the development of vacuole in the plant cell. a—meristematic cell and c—a mature cell.

PROCARYOTIC CELLS

A procaryotic cell, found in case of bacteria and blue green algae, differs in certain respects from eucaryotic cell found among plants and animals. In procaryotic cells, the protoplast can be differentiated into cytoplasm and incipient nucleus. The cytoplasm contains photosynthetic lamellae or chromatophores which are the sites of photosynthesis. Certain other lamellar structures or infolding of cell membrane called mesosomes are found to carry on respiration. The ribosomes lie free in the cytoplasm and are the site of protein synthesis.

Nucleus is not true as there is no nuclear membrane and nucleolus. The chromatin material lies free in the centre of cytoplasm in a form of tangled mass. Golgi complex, mitochondria and plastids are absent in procaryotic cells.

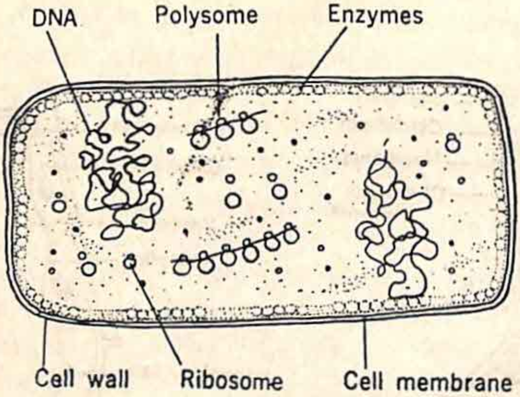


Fig. 1.6. A generalised diagram of a procaryotic cell (*Escherichia coli*) based on electron microscopic studies.

ANIMAL AND PLANT CELLS

The plant and animal cells can be distinguished from each other on the basis of certain characters. Externally an animal cell is bounded by a thin, delicate plasma membrane while a plant cell along with it has an additional and most distinguished structure—the cell wall. Cell wall is moderately rigid covering and is an extra cellular deposition. So cell wall is not found in animal cells. The animal cell also lacks plastids, i.e. colouring bodies participating in the manufacture of food. The plastids are the characteristics organelles of an autotrophic plant.

The animal cell contains centrosomes which play a significant role during cell division. Lysosomes are other structures found commonly in the animal cells and their presence in plant cell is doubted. Similarly vacuoles are of common occurrence in plants.

So, a plant cell has a cell wall, plastids and vacuoles as additional structures over an animal cell. Likewise, an animal cell has centrosome and lysosome as additional structures not commonly met in plant cells. However, the presence of centrosome in certain lower plants such as *Chlamydomonas* and *Spirogyra* is also known.

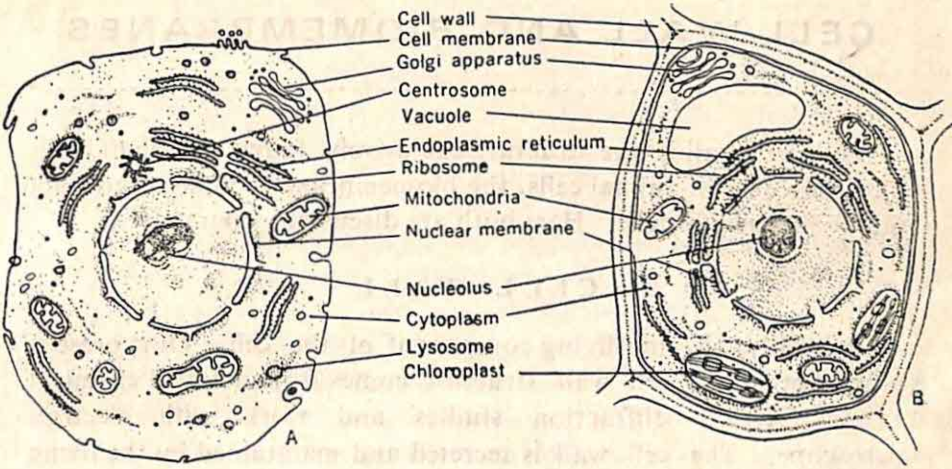


Fig. 1.7. Generalised comparative diagrams of typical animal and plant cells based on ultrastructure studies.

■ ■ ■

CHAPTER 2

CELL WALL AND BIOMEMBRANES

While cell wall is one structure exclusively found in plant cells and in no case in animal cells, the biomembrane is common to both animal and plants cells. Here both are discussed separately.

CELL WALL

Cell wall is the non-living component of the cell. Our present knowledge about cell wall structure comes from detailed chemical analyses, X-rays diffraction studies and work with electron microscope. The cell wall is secreted and maintained by the living portion of the cell called protoplast. The chief structural component of the cell wall is cellulose which is a chain of many thousands of sugar units. In addition to cellulose, pectic compounds, hemicellulose, lignin, suberin, cutin, protein etc. are also found in cell wall.

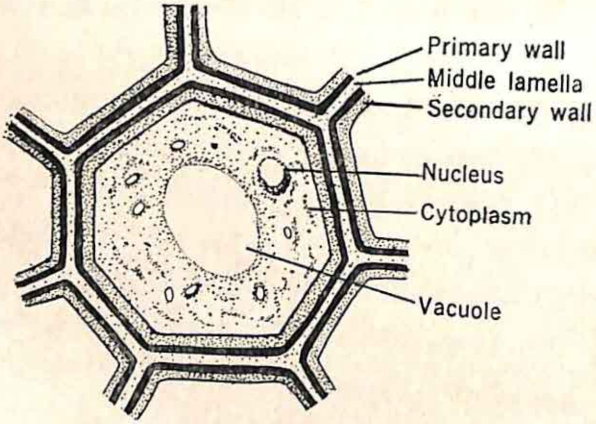


Fig. 2.1. A diagram of typical cell showing position of different wall layers.

A typical cell wall is composed of three different regions—(i) middle lamella, (ii) primary wall (1 – 3 µ thick and elastic) and (iii) secondary wall (5 – 10 µ thick and rigid).

Middle Lamella

The formation of this layer starts in the telophase stage of the cell division in the region of cell plate. It works as cementing layer between two daughter cells and is called middle lamella and consists of $Ca-$ and $Mg-$ pectates (composed of chains of hexuronic acids). So it's a common layer between the two cells and when with the help of proteolytic enzymes this layer dissolves, the two cells are separated. The loosening of mature ripe fruits is nothing but the result of the dissolution of middle lamella.

Primary Wall

Bordering middle lamella, the primary wall is the first product of cell wall synthesised by protoplast. In young enlarging cells, the primary wall remains thin and elastic becoming thick and rigid with the approach of cell maturity. The dry primary wall contains hemicellulose upto 50 %, cellulose upto 25% and smaller amounts of pectic substances, non-cellulosic polysaccharides, fats and proteins. Hemicellulose is composed of pentose polysaccharide and forms matrix of the wall in which cellulose microfibrils are embedded. Kerr (1951) observed that flexibility of primary cell wall during elongation is due to presence of pectic substances. Jenson (1960) reported that the cell walls of cortex and protoderm have low contents of pectic substances and hemicellulose while that of provascular cells have high contents of these substances.

Secondary Wall

The deposition of the products of cell wall synthesis by protoplast on the primary wall after maturation gives structural independence to the cell and provides rigidity to the wall. This newly deposited layer is called secondary wall.

The secondary wall mainly consists of cellulosic or varying mixture of cellulose and non-cellulosic material. On dry weight basis, a cotton fibre contains more than 90% cellulose. It may be modified through the deposition of lignin and other substances. As a result of localised deposition—annular, spiral, scalariform or ladder like, reticulate or net-like or pitted (simple or bordered) thickenings may be formed on the inner cell walls.

Organisation of Cell Wall

Structurally the cell wall may be thought of as a fine interwoven network of cellulose strands of varying complexity and size. According to Seigel (1962), the elementary fibrils or micelles are the smallest structural units of cell wall. These are composed of approximately 100 individual cellulose chains with a cross-sectional area of about 3000 A°. The molecules of cellulose, however, consist of long chains of about 3000 glucose units—(hexose) and in the cell wall these units are associated parallel to one another to form microfibrils. About 20 micelles with a cross-sectional area of about 62,500 A° form a microfibril. Micelles and microfibrils are visible under the electron microscope. A cotton fibre visible to naked eye has got as many as 1500 fibrils which contain about 7·5 × 10^8 individual cellulose chains. Albersheim (1965) has considered microfibril as the basic structural unit of cell wall.

In primary wall, the microfibrils are roughly transverse to the cell axis and do not show a longitudinal orientation at the cell corners. In the secondary wall, three distinct layers have been recognised with different arrangement of microfibrils. In the outer

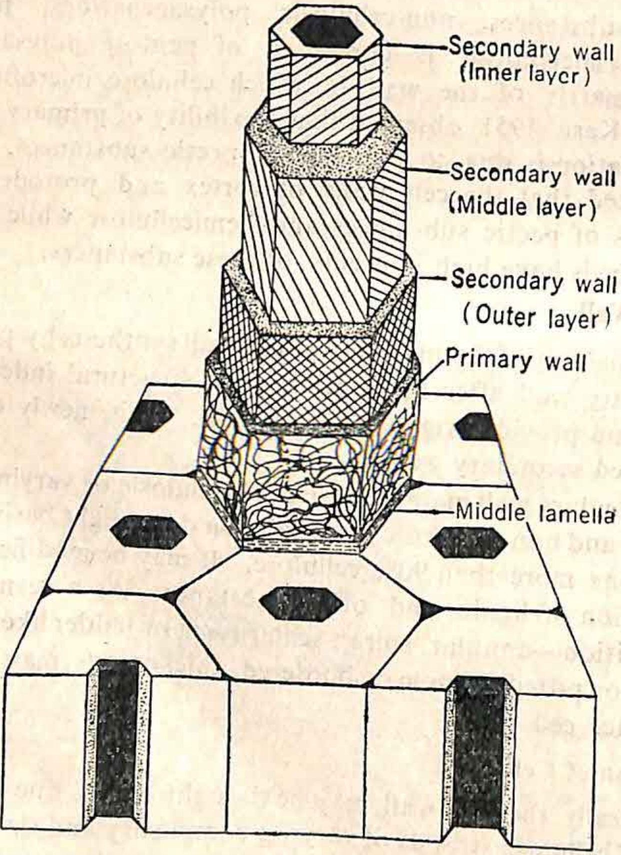


Fig. 2.2. Orientation of microfibrils in different layers of cell wall.

layer of the secondary wall two spirals of microfibrils may be seen forming a rather large angle with the cell axis. In the middle section, a steep spiral in addition to concentric rings of spirals can be observed and inner layer is thought to have a helical arrangement.

At places in the cell wall the deposition of material does not take place. These places are known as plasmodesmata (singular plasmodesma) through which contents of neighbouring cells remain in communication with each other.

FUNCTION OF CELL WALL

It performs two important functions. Firstly, it provides a mechanical support and gives a definite shape and protection and secondly, being hydrophlilic in nature, it is capable of imbibing water and helps in the movement of water and solutes towards protoplasm. It acts as permeable structure during absorption of minerals and solutes.

BIOMEMBRANES

Cell membrane may be more accurately referred as biomembrane since it is found as limiting structure of the entire cell as well as that of plastids, nucleus, mitochondria, endoplasmic reticulum, Golgi's apparatus, lamellae etc. in both plant and animal cells.

Composition of Membrane

Biomembrane has been found to consist of proteins and lipids, and, in some cases, polysaccharides, RNA and protein in varying proportions.

(i) MEMBRANE PROTEINS *: Three different classes of membranous* proteins—structural protiens, enzymes and carrier proteins have been found in many cases.

(a) *Structural proteins* : Constituting the backbone of membrane, the structural proteins are extremely lipophilic that display little catalytic activity. In such proteins, from one fourth to one third of the amino acid residues show α-helix secondary conformation while the remainder forming the random coils (Lenard and Singer, 1966). So it will not be untrue to admit that plasma membrane consists largely of structural proteins and studies made on that of *Mycoplasma* suggest that more than a single variety of structural protein is present in a membrane.

(b) *Enzymes* : In many cases, the major components of membrane are catalytic proteins or enzymes that vary considerably in structure and amount. Such proteins specify the physiological function of membrane. For example, the marker emzymes of endoplasmic reticulum include glucose 6-phosphatase and for mitochondria glycerol phosphate dehydrogenase and cytochrome oxidases.

(c) *Carrier proteins* : Carrier proteins of membrane have not been as yet well defined. Their molecular weight is close to $3{\cdot}2 \times 10^4$ daltons in *Salmonella typhimarium* where it acts as sulfate carrier. Similarly a glucose binding factor in *Escherichia coli* with mol. wt. $4{\cdot}5 \times 10^4$ dalton's has also been isolated.

Both carrier and structural proteins are of low molecular weights. It is interesting to note that in certain cases these proteins may have dual functions.

(ii) MEMBRANE LIPIDS : The amount of lipids present in membrane vary from 30 percent (in mitochondria) to 75 percent (in myelin) of the total dry masses of biomembranes. However, major lipids are polar lipids and sterols. In animal membranes, the polar lipids present are about 70 to 85 percent consisting of primarily phosphatidyl choline, phosphatidyl ethanolamine, sphyngomyelin, phosphatidyl serine and cerebrosides in varying proportions. The mitochondrial membrane is especially enriched with diphosphatidyl glycerol.

The physical properties associated to membrane are mainly imparted by polar lipids which are amphipathic molecules. The formation of minute spherules to seal themselves from the mechanically disrupted larger complexes of endoplasmic reticulum is due to the same property.

(iii) MEMBRANE POLYSACCHARIDES AND GLYCOLIPIDS : The amount of polysaccharides present in biomembranes is extremely low. Cerebroside glycolipids are present in myelin and gangliosides in many other membranes. Gangliosides resemble with the phospholipids in that they have a hydrophobic chain with large polar portion and terminating into sialic acid (free carboxyl group). The polar chain also contains N-acetyl galactosamine, glucose and galactose. Stein (1967) observed that gangliosides are involved in transfer of ions in erythrocytes.

However, the molar ratio of cholesterol, phosphatides and glycolipids is close to 1 : 1 : 2 in certain cases of animals.

(iv) MEMBRANE RNA : Considerable evidences for the occurrence of RNA as integral components of biomembrane have been observed. Bal (1966) while working on *Allium cepa* noted that RNA is an integral component of nuclear membrane, endoplasmic reticulum, plasma membrane and cell wall. The exact location of RNA is, however, still not clear. Shapot and Pitot (1966) have detected small amount of RNA in the smooth as well as rough membrane of endoplasmic reticulum. RNA has also been observed in the membranes of chloroplast and mitochondria.

(v) MEMBRANE DNA : DNA has been detected in the membranes of mitochondria, chloroplast, yolk granules and plasma membranes. In first three cases at least some of DNA is present in circular form and is easily separable. It may not, therefore, be considered as integral component of membrane. However, the presence of DNA in the mitochondrial membrane has been demonstrated.

(vi) SOME OTHER COMPONENTS : Other components playing vital roles in membrane physiology have also been detected such as certain metals (*Ca*, *Zn*, *Mg*), coenzymes, porphyrins (hematin, chlorophyll) etc. are found present in biomembranes.

Models of Biomembrane Structure

Of the many models proposed a few are discussed here as follows.

1. Danielli-Davson Model : Upto 1936 this model dominated in the mind of biologists, biochemists and biophysicists as it answered, to some extent, the problem of permeability of cells. The chief features of the model included one or more bimolecular leaflets of phospholipids sandwitched between two layers of globular proteins in essentially separate and continuous phase. The phospholipid molecules are closely packed and standing perpendicular to the surface of membrane with their polar heads adjacent to the surface protein.

2. Unit membrane hypothesis : Electron microscopy helped in finding membrane limiting several other organelles within cell besides the cell membrane which led Robertson (1964) to propose his unit membrane hypothesis. The model, however, did not differ greatly from that of Danielli-Davson model. It restricted the number of bimolecular lipid leaflets to one and mucoprotein on outside face and non-mucoid protein on the inside face of the membrane. In both these models, the lipid and protein molecules are held electrostatically, the former being negatively charged while latter positively charged. In simple terms, the model can be explained as follows.

Two molecules of lipids are sandwitched between two molecules of proteins. In this sandwitch the lipid molecules are said to have a remarkably orderly arrangement in which the fatty acid (lipophilic) tails face one another along the double row. The hydrophilic heads face outwards and appear to be at least partially embedded in the outer protein layer. Each outer protein layer is almost 20 A° thick and the lipid in between is 35 A°, thus making the total sandwhich 75 A° thick. At places, the lipid layers are penetrated by pores of 7-10 A° size which are lined with positively charged groups.

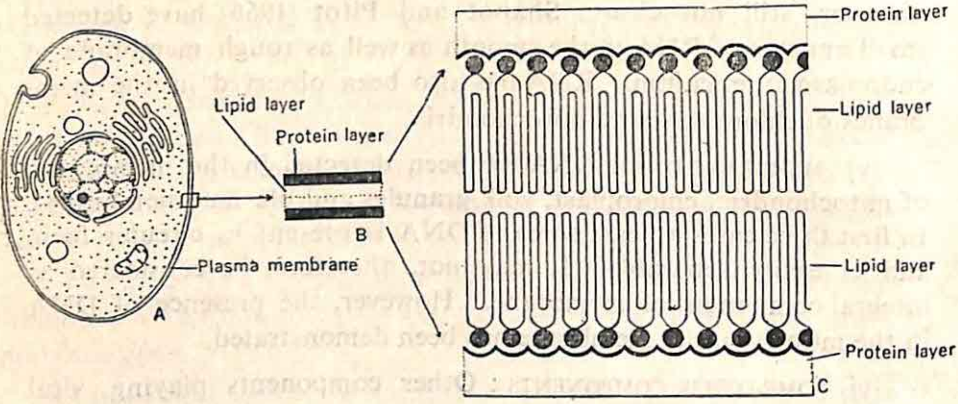


Fig. 2.3. Structural details of plasma membrane. A—as seen under ordinary magnification of electron microscope; B—a little more enlarged about 50,000 times; and C—a detailed structure showing sandwitch arrangement.

Glauert and Lucy (1968) stated that phospholipid (cholesterol and lecithin) molecules are not necessarily present in two layers but often in a single layer (fig. 2.4 A and B). The lipid material assumes the form of small spheres called micelle. The hydrophilic parts of both cholesterol and lecithin occur at the periphery of the micelle while lipophilic parts directed centrally.

3. *Kavanau's lipid-pillars* : J. L. Kavanau (1966) proposed modifications earlier to both these models considering the physical and physiological properties of the membrane such as selective permeability, contractibility etc. This model visualises the lipid components being oriented in descrete pillars of lipid micelles under certain conditions and as nearly continuous flattened discs under

other conditions. The gaps between the micelles serve as water pore for the ion penetration. The protein is however present on both sides of membrane and consists of two types (i) matching the areas delimited by the bases of the pillars and (ii) filling in the spaces among these. Membrane in the pillar region must be thick and in the disc region thin. The surface of each pillar or disc is comprised of the polar heads of phospholipids and the anterior of these is filled with non-polar tails of phospholipids and non-esterified sterols. Kavanau postulated that rhythmic raising and lowering of pillars by cyclic changes may be responsible for intracellular movement and cytoplasmic flow.

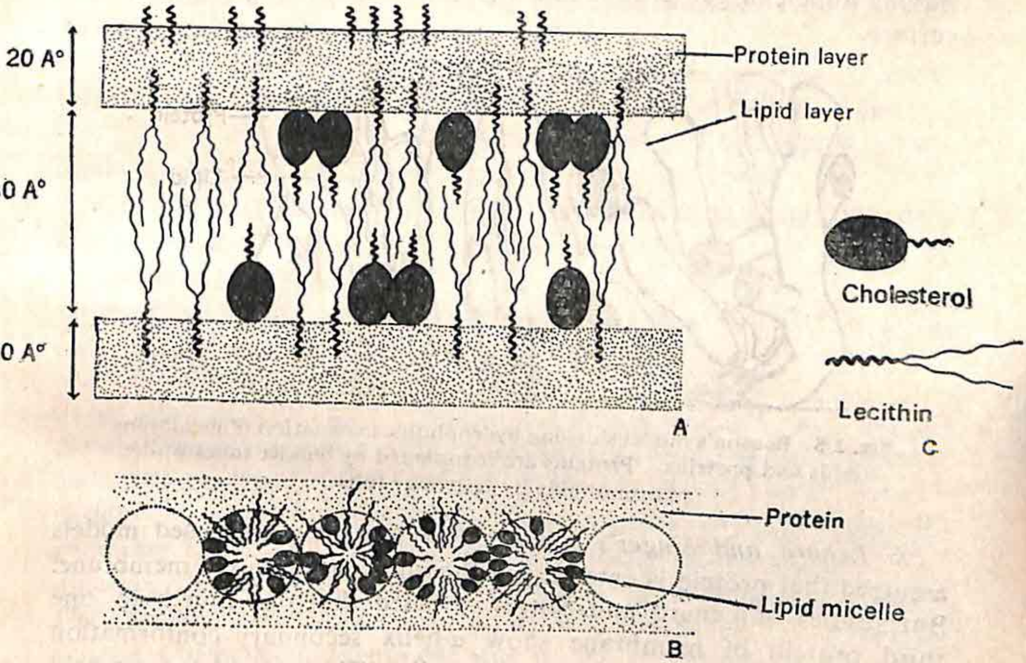


Fig. 2.4. The proposed model of biomembrane. A—cholesterol and lecithin in the centre showing two rows of lipid; B—the lipids are actually arranged in a single row in the form of micelle and C—molecular representation of cholesterol and lecithin.

4. *Hydrophobic binding models* : From the time Benson (1966) while working on chloroplast membrane and Fleischer and Brierley (1961, 66) working on mitochondrial membrane observed that in a membrane binding between lipid and protein is of hydrophobic rather than electrostatic in nature, the unit membrane concept has been

challenged. Green and Perdue (1966) have also supported this view and visualised that the union between two (protein and lipid) is too tight to be explained by electrostatic concept. However, these authors visualise a strong hydrophobic association among the hydrocarbon side chains of amino acid residues of membrane proteins and the hydrocarbon tails of the lipids.

5. *Benson's model* : Based on the study of chloroplast membrane, Benson (1966) proposed that the lipids of membrane sub-units are bound by hydrophobic association of their hydrocarbon tails with complementary hydrophobic regions *within the interior* of the protein leaving ionic charged groups of the phospholipids on the membrane surface.

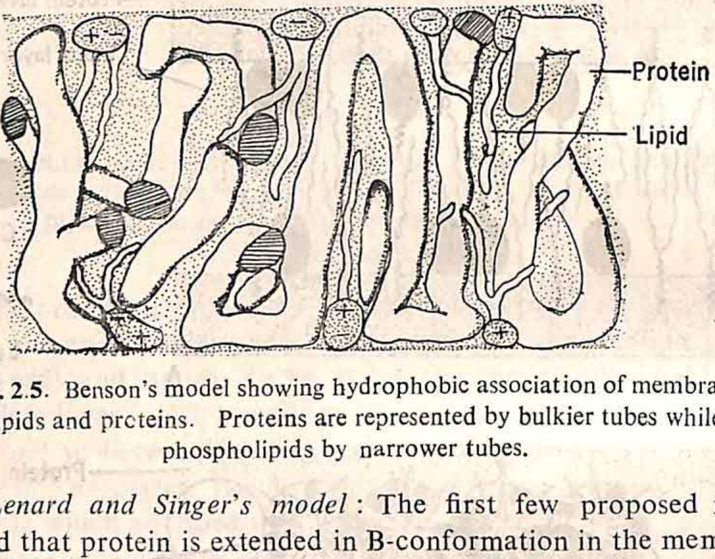


Fig. 2.5. Benson's model showing hydrophobic association of membrane lipids and proteins. Proteins are represented by bulkier tubes while phospholipids by narrower tubes.

6. *Lenard and Singer's model* : The first few proposed models assumed that protein is extended in B-conformation in the membrane. But studies of Lenard and Singer revealed that one fourth to one third protein of membrane show α-helix secondary conformation while the remainder exists as random coils. These structures are held to each other or to the tails of membrane lipids by hydrophobic interactions and the helical structure is stabilised by the hydrophobic interactions.

7. *Mosaic membrane concept* : Green and Perdue (1966) proposed that membrane comprises of repeating units which are assymmetrical in a direction perpendicular to the surface and these repeating units in a membrane may not be identical. They have compared these units with elementary particles of mitochondria that extend outward from the membranes comprising cristae. In their own

words "in any given membrane layer the repeating units are all complementary in form......but not in composition. In the inner membrane of mitochondrion there may be as many as ten different species of elementary particles each chemically and enzymatically unique." Branton's (1969) recent study also elucidates the same fact that cell membranes possess particles which are embedded in their matrix and can protrude out at one or both of their surfaces. These vary in number and arrangements and are bigger in the plasmalemma than in the tonoplast.

8. *Composite model* : Schjeide and Lin (1970) visualise that any model proposed to explain the structural organisation of biomembrane should exhibit the following characteristics and then it should be called composite model.

(i) The different components can be fixed in physical orientations ranging from lamellar sheets to aggregation of microtubules.

(ii) The integral components may include proteins, lipids, glycolipids, carbohydrates, RNA and DNA.

(iii) The association between lipid and proteins is mainly of hydrophobic nature. However, corpuscular units may be linked by electrostatic bonds.

(iv) The polar groups are oriented towards the surface while hydrophobic groups are mainly located internally.

(v) They are usually mosaics and often asymmetric.

9. *Fluid-mosaic model* : Singer and Nicolson (1972) summarised the modern concept concerning biomembrane. The model accommodates the presence of a bilayer of lipid molecules containing a variety of protein molecules. Some of the protein molecules penetrate right through the lipid layer, whilst others do not. The function of lipid layers is to provide the basic permeability barrier. The proteins are concerned with two functions, i.e. with the transport of molecules from one side of the membrane to the other and with the various enzymatic activities of the membrane.

Besides, the recent studies (Lee, 1975) on biological membrane have suggested that membrane should now be pictured as highly fluid rather than as a highly ordered and static structure. Applying most sophisticated techniques to the biomembrane studies, it has been found that the following motions are possibly found in it—

(i) internal motion within each lipid molecule.

(ii) lipid molecule might diffuse laterally.

(iii) lipid molecules may be able to rotate rapidly as a whole about their long axes.

(iv) transfer of lipid molecules from one side of the bilayer to the other. It is referred as 'flip-flop' movement.

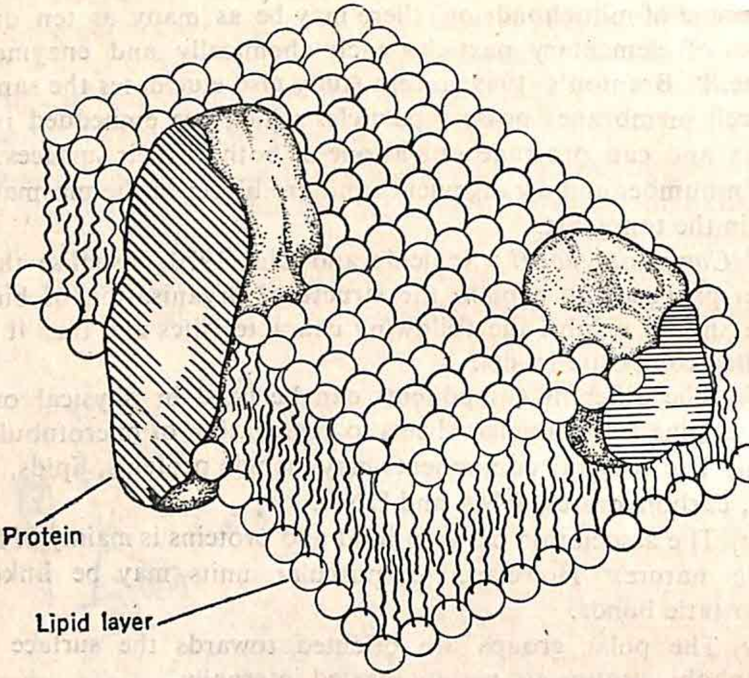


Fig. 2.6. Model of biomembrane as proposed by Singer and Nicolson (1972).

SOME SPECIAL STRUCTURES OF BIOMEMBRANES

Microvilli and desmosomes are considered as the specialisation of the cell surface.

Microvilli

Invaginations of the plasma membrane into the cytoplasm are seen in many kinds of cells. One of the good examples is found in the cells of intestinal epithelium. These folds are called microvilli. The microvilli are generally 0·6 to 0·8μ in diameter enriched

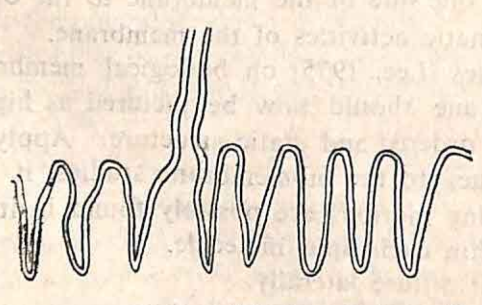


Fig. 2.7. Microvilli—invaginations of plasma membrane.

Date 30.10.98
Accn. No 9755

with dense cytoplasm containing numerous mitochondria and these provide considerably increased surface for absorption. There may be 3000 microvilli in a cell and nearly 200,000,000 in a mm^2 area. Microvilli are also known from mesothelial cells, epithelium of gall bladder, uterus, liver cells etc.

Desmosomes

Certain closely organised cells show the direct connections between the membranes during spermatogenesis of *Hydra*, *Drosophila*, rat, cat, rabbit, monkey and man (Fawcet, 1961) by developing the thickening on the inner surface of adjacent plasma membranes. From such thickenings arise five fibrils called tonofibrillae which radiate and pass through the bridge and enter the interior of the cell. Now the whole structure is called desmosomes. Thus, this modification is restricted to the inner layer of the membrane while outer membrane always remains unaffected. Chemically, desmosomes are not different from cell membrane but the protein, called stromatin, has a high molecular weight and is fibrous in nature (see fig. 2.8).

FUNCTIONS OF BIOMEMBRANES

Some of the known important functions of the membrane may however be summarised as follows :

(i) It acts as permeability barriers which control and coordinate rates of substrate transfer and diffusion.

(ii) It acts as cytoskeleton providing mechanical frames on which enzymes can be specifically oriented.

(iii) It acts as a vehicle for transport of substances from one organelle to another, from inside to the outside the cell and from outside to inside the cell. Mitchell (1963) suggested an explanation for the entry of large molecules–like glucose by the process called *translocation*. The molecules to be transported fits into a site of special configuration (probably in the region of pore) on the outside of membrane and then is carried through the membrane and released on the other side. It is believed that carrier site is highly specific for each molecule and that only when the site is filled, the carrier performs its function. The mechanism is so well suited that it hardly requires intervention of any energy utilising reaction.

(iv) It acts as a source of information of various kinds.

(v) It acts as elements capable of synthesis of various macromolecules.

(vi) The functions like phagocytosis and pinocytosis are also associated to it.

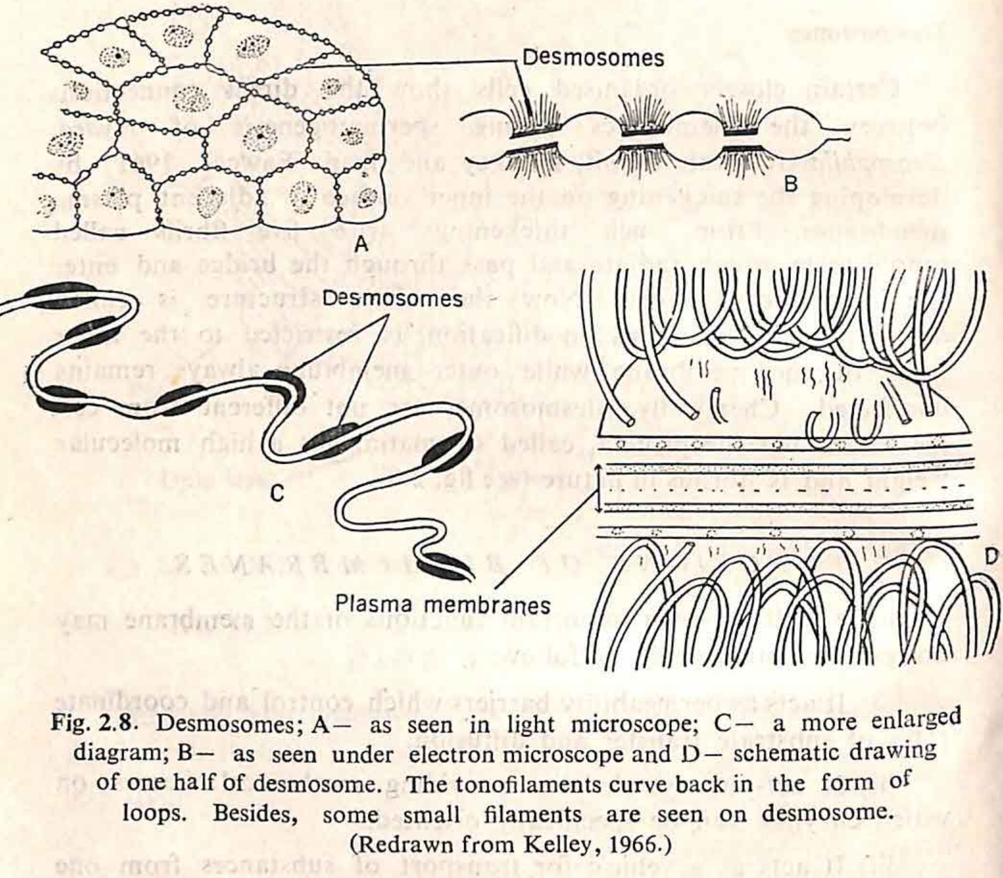


Fig. 2.8. Desmosomes; A— as seen in light microscope; C— a more enlarged diagram; B— as seen under electron microscope and D— schematic drawing of one half of desmosome. The tonofilaments curve back in the form of loops. Besides, some small filaments are seen on desmosome. (Redrawn from Kelley, 1966.)

Phagocytosis

In certain cases, the food is obtained by ingestion of solid particles through plasma membrane. The phenomenon is called phagocytosis after a Greek word *phagein* which means to eat. Such examples are witnessed among majority of protozoans and among certain cells of metazoans.

The mechanism involved in phagocytosis in protozoans is intimately associated with amoeboid motion and the larger particles are ingested in a food vacuole formed with the help of pseudopodia that progressively surround them. However, among metazoans, it generally serves as a means of defence rather than involved in cell nutrition. The foreign particles like bacteria are absorbed at the surface of membrane and later into the cytoplasm by infolding of plasma membrane.

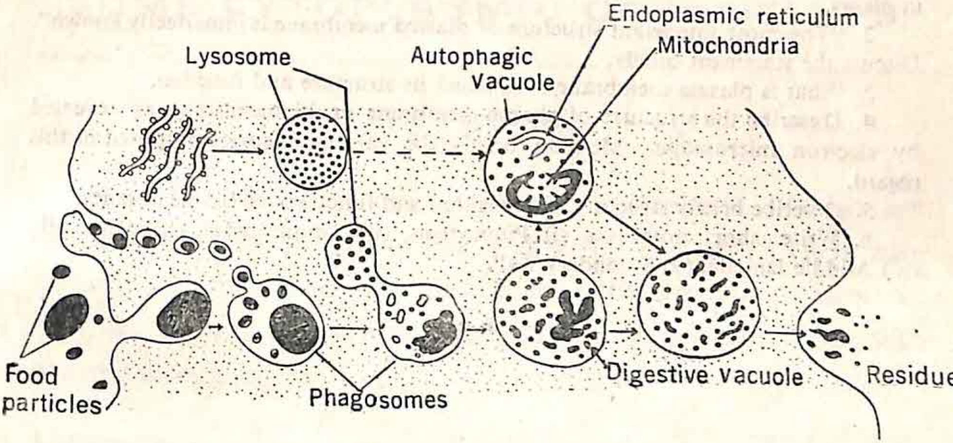


Fig. 2.9. Demonstration of phenomenon of phagocytosis. The food is ingested by forming invagination in cell. It thus forms a phagosome which on fusion with lysosome forms a digestive vacuole. The food is digested and the digestion products diffuse in cytoplasm while indigestible matter is ejected out in the form of residues. The diagram also shows digestion of endoplasmic reticulum and mitochondria, which too are digested similarly.

Pinocytosis

Lewis (1931) observed the ingestion of fluid vesicles by the living cells. This process he called pinocytosis. The substance is first absorbed at the surface and then by the infolding of membrane a a vesicle is formed which is soon pinched off and transported to the other region of cell. Mast and Doyle (1934) demonstrated the entrance of protein by this method in *Amoeba*.

(vii) Cell membrane helps in the active transportation of movement of ions and molecules across the biomembrane. Following active transport, Stein (1969) suggested that movement of molecules or ions in a direction opposite to that of a prevailing electrochemical gradient occurs. There may be several possible mechanisms of active transport such as formation of substrate enzyme complex, presence of some carriers, involvement of metabolic energy etc. but they are beyond the scope of the book.

GENERAL QUESTIONS

1. Write an illustrated account of structure, function and origin of cell wall in plants.
2. "The most important structure of plasma membrane is imperfectly known". Discuss the statement briefly.
3. What is plasma membrane ? Discuss its structure and function.
4. Describe the structure of plasma membrane or biomembrane as revealed by electron microscope. Mention briefly the various theories proposed in this regard.
5. Describe briefly structure, composition and functions of biomembranes.
6. Write short notes on (i) Pinocytosis, (ii) Desmosomes, (iii) Microvilli, (iv) Middle lamella, (v) Secondary wall.

■ ■ ■

CHAPTER 3

SOME CYTOPLASMIC ORGANELLES

PLASTIDS

Found lying freely in the cytoplasm of plant cell, plastids are discoid organelles about 5μ in dia. and 3μ in thickness. There may be great variations too in the size. These either contain pigments or have the property to develop them. Three different types of plastids are well recognised.

Chromoplasts

These are coloured plastids which contain variously coloured pigments other than photosynthetic ones. They can arise either from chloroplasts as in petals which are initially green but become coloured subsequently or from leucoplast as in carrot roots. No perfect structural organisation of these plastids is fairly known. Some times these colouring pigments are present in vacuolar sap as in petals. In this form they provide resistance to plant against thermal fluctuations. Also they are cold resistants and that is why variously coloured flowers are produced in temperate regions and during winters in tropical regions. The lower surface of *Nymphaea* and newly produced buds during winter are coloured. At times, it is also believed that their presence in flowers serves for attracting insects thus helping in pollination and propagation.

Leucoplasts

These plastids contain no visible pigments. Some times they are white in colour. They are involved in the storage of various kinds of reserve food material and are thus accordingly named. If they are involved in the synthesis and storage of starch, they are called **amyloplasts**, if proteins called **aleuroplasts** and if oil **elaioplasts**. Of these amyloplasts are important and resemble to proplastids as they contain fewer lamellae being capable of forming thylakoids in presence of light.

Chloroplasts

These were firstly observed by Anton Von Leevwenhoek in the later years of the seventeenth century and from the time Sachs (1862) observed starch grains inside chloroplasts during photosynthesis, these are believed to be sites of photosynthesis.

The chloroplasts are small, green bodies present in the cytoplasm of higher plants and green algae. The corresponding organ of red and brown algae are called chromoplasts whereas blue green algae and certain bacteria such as green and purple types contain neither one. The pigments of blue green algae are more or less distributed evenly in chromatoplasm, preferable in the structure called phycobilisomes. In case of chromoplasts and chloroplasts, the pigments are concentrated in them. The chloroplasts in algae exhibit a remarkable variation in size and shape, e.g. star-shaped, band-shaped, disc-shaped, or giants with linear dimensions. *Chlorella* contains a single bell-shaped chloroplast; *Chlamydomonas*, cup-shaped; *Ulothrix*, girdle-shaped; *Oedogonium*, reticulate; *Spirogyra*, band-shaped and *Zygnema* stellate-shaped chloroplasts. A characteristic feature of these chloroplasts is the presence of pyrenoids. The pyrenoids are irregular-shaped bodies composed of viscous mass of protein surrounded by starch platelets.

The chloroplasts of higher plants are mainly found distributed in palisade and spongy tissue of leaves. They may be flat ellipsoids or disc-shaped and in average 3-10 μ across. Haberlandt (1882) found an average of 36 chloroplasts in each palisade cell and 20 in each spongy parenchyma cell. However, this number may vary a lot.

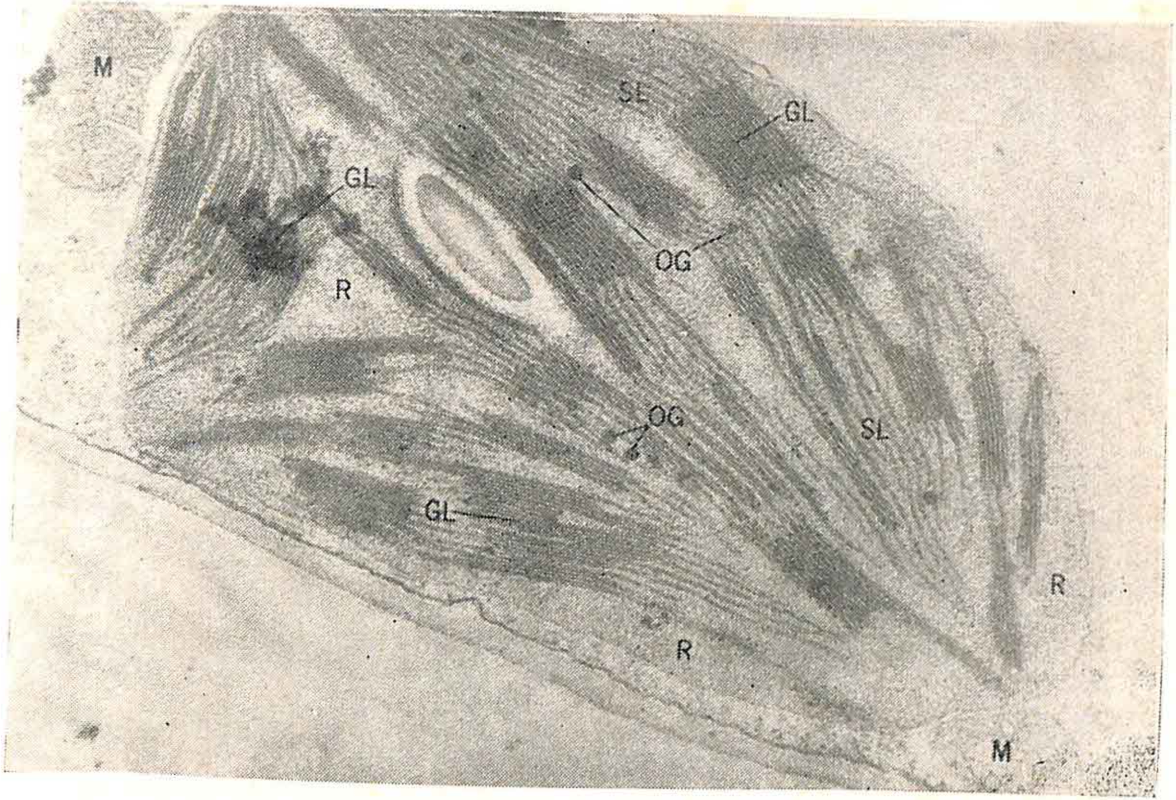


Fig. Pl. 3.I—Electron micrograph of chloroplast. Gl=grana lamellae, SL—stroma lamellae, M=mitochondria, OG=osmophilic granules, R=ribosomes (present in stroma). (With courtesy from *Dr. E. Weier*).

In case of *Mnium* (a moss plant), an average number of 106 chloroplasts were found in each cell.

(A) STRUCTURE OF CHLOROPLASTS : Every chloroplast contains mainly four types of pigments : chlorophyll *a*, chl. *b*, xanthophyll and carotene. They are thought to be arranged orderly.

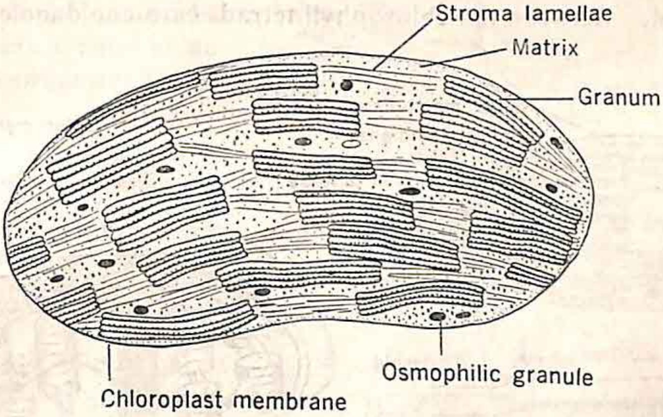


Fig. 3.1. Chloroplast, a diagram based on electron micrograph.

Each chloroplast remains surrounded by two unit membranes of lipoproteinaceous nature. Each unit membrane is composed of 2 lipid molecules arrested in between 2 protein molecules. The external surface of outer chloroplast membrane is smooth whereas inner membrane is thrown into lamellated structure showing two distinct regions—

(*a*) *Stroma* : In this portion of chloroplast, lamellae are not compactly arranged but are loose. The lamellae which are found in this region are called stromatic lamellae or stroma lamellae. The stroma is the main site for the dark reaction. However, ribosomes are also found in the stroma which means that chloroplasts can synthesise some of their protein.

(*b*) *Grana* : Grana are the most important but complicated portions of the chloroplast. Lamelle in this region are compactly arranged just like stack of coins, i.e. grana contain lamellae in a series one upon another placed back to back. The pigments of chloroplast are actually distributed in this region. How are the pigment molecules arranged in them ? Wolken and Calvin tried to explain this. They proposed that the chloroplast is a lamellar structure composed of lipid and aqueous protein layers. The chlorophyll molecule is 'polar' consisting of a hydrophilic porphyrin head and a lipophilic phytol tail. The porphyrin parts extend into the aqueous protein and the phytol part into the lipid layers. Wolken (1961) while working on *Euglena* further proposed that chlorophyll molecules are arranged in tetrads and in such a way that isocyclic rings of porphyrin heads face each other and phytol tails remain separated. Between the chlorophyll tetrads carotenoid molecules are found.

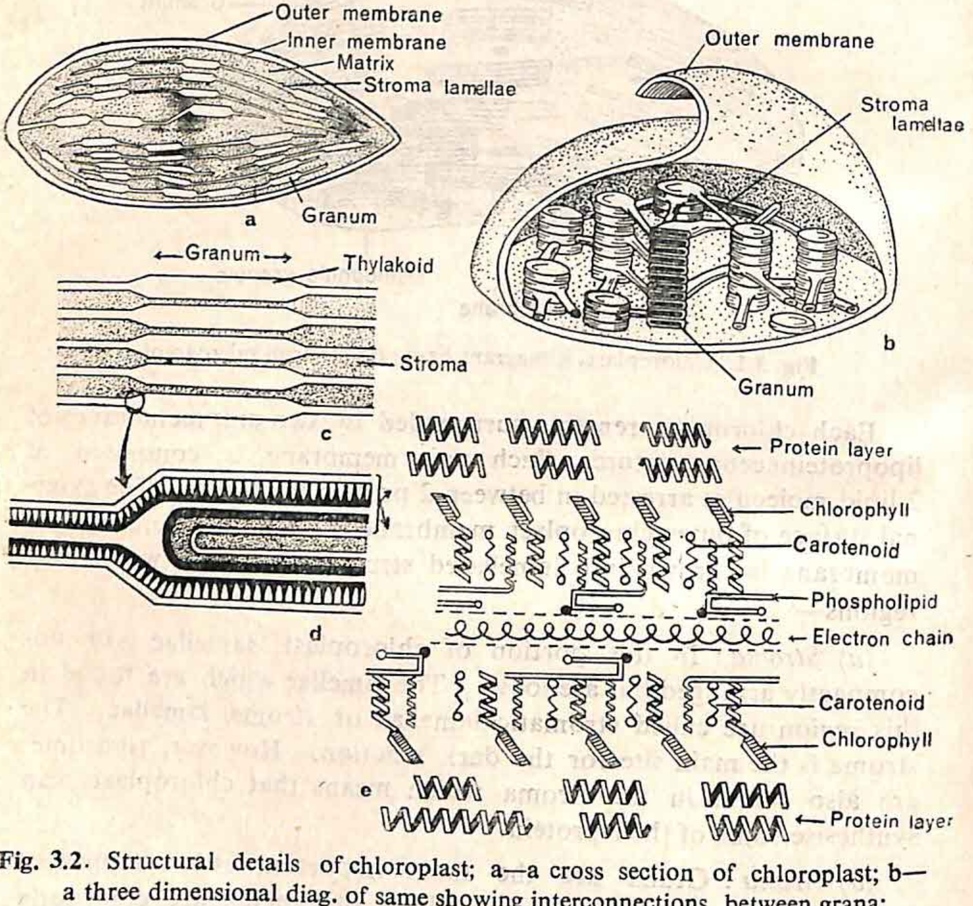


Fig. 3.2. Structural details of chloroplast; a—a cross section of chloroplast; b—a three dimensional diag. of same showing interconnections between grana; c—a portion of granum enlarged; d—lamellae shown enlarged from c; e—diagramatic molecular arrangement of different constituents of chloroplast.

Calvin gave a model similar to Wolken and said chlorophyll molecules are found in monomolecular layer, the porphyrin head facing towards proteinaceous portion and phytol tail toward lipid portion. In later years Park and Beggin (1964) studied the chloroplast lamellae and found that these form pocket-like structures called thylakoids. In thylakoids, they observed some small rounded structures arranged in a monolayer fashion on thylakoid bodies. These structures are called quantasomes. The surface arrangement of the quantasomes give a cabble-stone appearance. On the basis of their internal structures, quantasomes can be divided into two clear regions *first*--the peripheral region enriched with pigment molecules and *second*—the middle region or the energy centre. In these specialised quantasome structures, only a single molecule of pigment can absorb energy at a time and all other molecules support the action of light reaction of photosynthesis. That's why quantasome is also called photosynthetic unit.

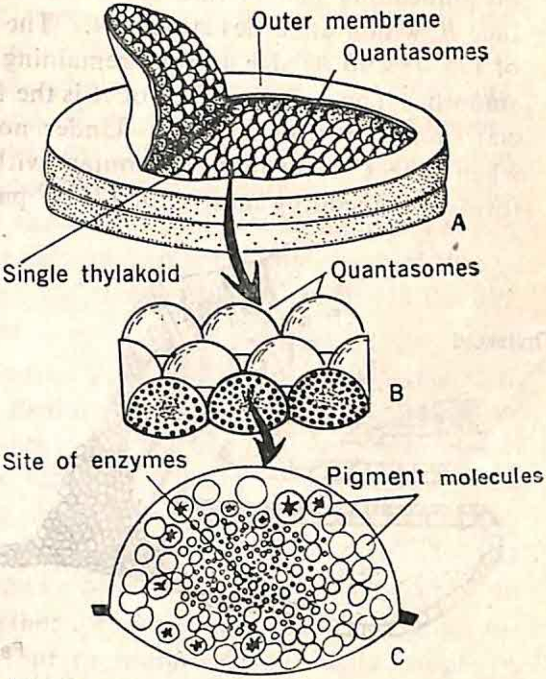


Fig. 3.3. Structure of chloroplast. A—a three dimensional structure of single granum thylakoid; B—a portion of same showing a few quantasomes and C—a cross section of a quantasome.

(B) ULTRASTRUCTURE OF CHLOROPLAST LAMELLAE : Details of chloroplast structure have been revealed by using the efficient possible modern techniques such as freeze etching and electron microscopy. Two important models proposed to explain the recently established ultrastructure are discussed here.

Branton and Park model : Branton and Park (1967) observed that the thylakoid membrane of chloroplast lamellae is composed of two sub-units appearing as two different particles in the membrane surface in electron micrograph. They noted that organisation of these particles is so as to involve three different faces of the membranes. The outer surface is face *A* which is seldom revealed. It consists of an embedding matrix that surrounds the tops of particles found on face *B*, which underlies surface *A*. The surface *B* contains particles of $175\ A^{\circ} \times 90\ A^{\circ}$ size and the remaining portion of the surface *B* is smooth. The under surface of *A* is the face *C* which contains particles of $110\ A^{\circ} \times 90\ A^{\circ}$ size. Under normal conditions, they stated, when face *C* is brought in contact with face *B*, the $110\ A^{\circ}$ particles form an embedding matrix for $175\ A^{\circ}$ particles composing face *B*.

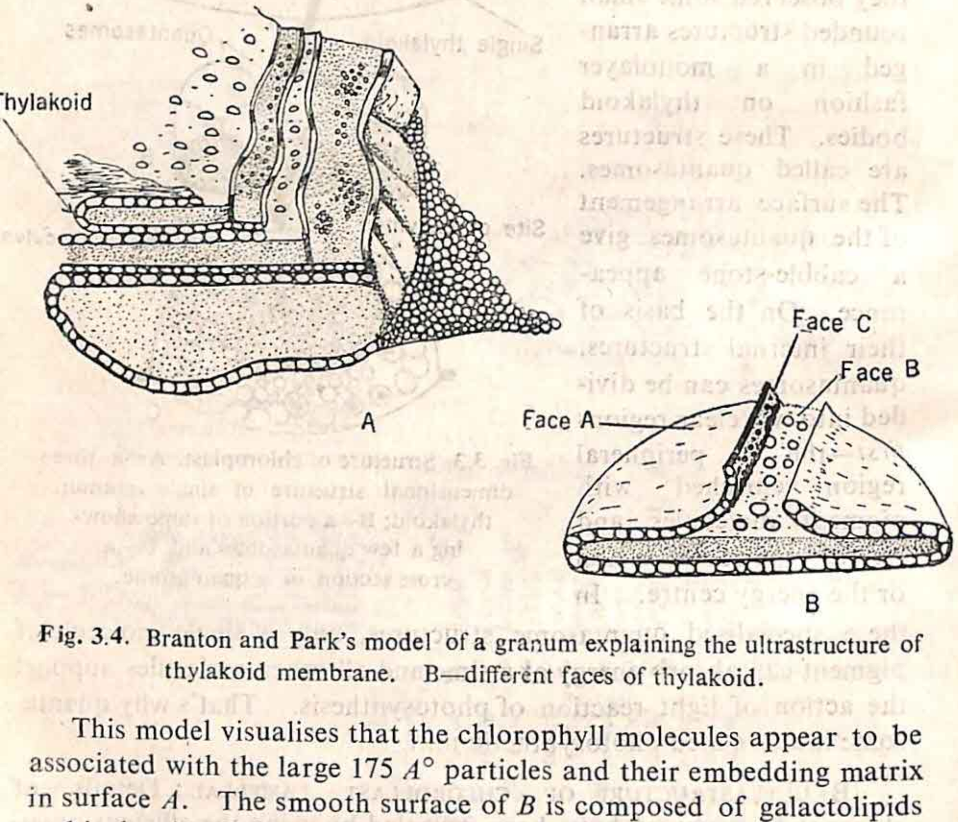


Fig. 3.4. Branton and Park's model of a granum explaining the ultrastructure of thylakoid membrane. B—different faces of thylakoid.

This model visualises that the chlorophyll molecules appear to be associated with the large $175\ A^{\circ}$ particles and their embedding matrix in surface *A*. The smooth surface of *B* is composed of galactolipids and is devoid of chlorophyll. Park (1966) and Branton (1968) have suggested that these $175\ A^{\circ}$ particles might be identical to quantasomes.

Weier and Benson model: This model is little complicated and a typical. The chloroplast membrane is composed of lipoprotein subunits each consists of a protein core coated on the outside with lipid coat. The model visualises the chloroplast membrane being composed of a single sheet of lipoprotein subunits. The two membranes are closely appressed. These appressed areas are called partitions. They have recognised three electron transparent hydrophilic spaces—

(i) the space enclosed by the granule membranes called loculi, (ii) the space enclosed by fret membranes (stroma lamellae) called fret channels and (iii) the space that contains the stroma and thylakoids in the chloroplast. The hydrophobic chlorophyll molecules are located in the appressed region of the partition. These molecules may be entirely contained within the subunits or their more hydrophilic porphyrin heads may occupy the area between the subunits while molecule tails buried in the anterior of the subunits. That is to say, the chlorophyll molecule arrangement within the partition is irregular and even chlorophyll molecules may be present in the fret channels but within the subunits.

(C) THE PHOTOSYNTHETIC UNITS : Park and Beggins (1964) isolated the photosynthetic units as distinct morphological structures from the chloroplast lamellae of spinach chloroplast and found to possess a molecular weight of about two million and contain approximately 230 chlorophyll molecules. They called these photosynthetic units the quantasomes. The quantasomes are about 180 $A° \times 160$ $A°$ and 100 $A°$ thick, i.e. size of particles close to 175 $A°$ particles of Branton and Park model. They further propose that each quantasome appears to be composed of four or more subunits which might be identical to subunits present in the partition of Weier and Benson model. The chemical composition of a quantasome is given as follows (as given by Park, 1965 : only the number of important molecules are given).

Chlorophyll *a* (160), chl. *b* (70), *B*-carotene (14), leutin (22), violaxanthin (6), neoxanthin (6), plastoquinone *A* (16), plastoquinone *B* (8), plastoquinone *C* (4), vitamin *K* (4), phospholipids (116), digalactosyldiglyceride (144), nitrogen atoms as protein (9,380), manganese (2), iron (12), copper (6).

(D) CHEMICAL COMPOSITION OF CHLOROPLAST : An average chloroplast reveals the following chemical composition.

Protein	40-50 percent
Phospholipid	23-25 ,,
Chlorophyll	5-10 ,,
Carotenoids	1-2 ,,
RNA	5 ,,
DNA	as fragments

Magnesium in the leaf occurs at a frequency of 2-3 percent of the total ash.

Willstater and Stole demonstrated the presence of four different pigments in higher plants. Both chlorophylls are green in colour whereas carotene and xanthophyll are orange and yellow respectively. The chlorophyll *a* and chlorophyll *b* occur in the ratio of 3 : 1 but chlorophyll and xanthophyll 2 : 1. The chemical composition of chlorophyll *a* is $C_{55}\ H_{72}\ O_5\ N_4\ Mg$ and of chl. *b* is $C_{55}\ H_{70}\ O_6\ N_4\ Mg$. Each chlorophyll molecule consists of two parts :

(i) *a tetrapyrrole ring* : Composed of four pyrrole nuclei held together by intermediate atomic groupings is called the porphyrin head. It is 15 $A°$ in length and breadth both. A magnesium molecule is found enclosed in the complex ring of pyrroles.

(ii) *a phytol tail*—It's a long chain of carbon and hydrogen of 20 $A°$ in length. Its chemical formula is $C_{20}\ H_{39}\ OH$.

Two types of carotenoids—the carotene ($C_{40}\ H_{56}$) and the xanthophyll ($C_{40}\ H_{56}\ O_2$) are other important pigments in the chloroplast. They act as accessory pigments.

(E) FUNCTION OF CHLOROPLASTS : Chloroplasts are the sites of light reaction of photosynthesis but a few stages of dark reaction also occur within them. However, some exact functions are summarised here.

(i) Photolysis of water (break down of water into $H+OH$ ions but the term photolysis is not used now).

(ii) Reduction of $NADP$ or production of $NADPH+H^+$ which acts as final hydrogen donor during the reduction of carbon dioxide to carbohydrate.

(iii) Photophosphorylation, i.e. production of ATP molecules utilising light energy.

(iv) The stroma region provides the main site for the dark reaction of photosynthesis.

(v) Besides their participation in photosynthesis, in certain cases they are metamorphosed into chromoplasts such as in fruits of tomato.

(F) ORIGIN OF CHLOROPLASTS : Many hypotheses have been proposed but only the following have been included.

(i) *Origin from proplastids* : The proplastids are fine globular double membrane particles and during the course of development the formation of infoldings from inner membrane occurs parallel to the surface. They are further differentiated into stroma and lamellae. Some biologists hold strong opinion about the nuclear origin of

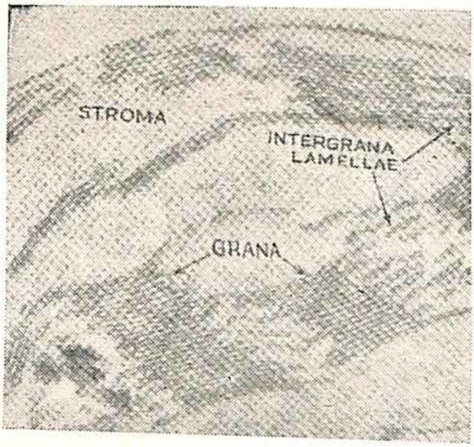


Fig. Pl. 3. II—Highly enlarged portion of microphotograph of chloroplast. The grana and stroma are quite clear.
(With courtesy from *E. Weier*).

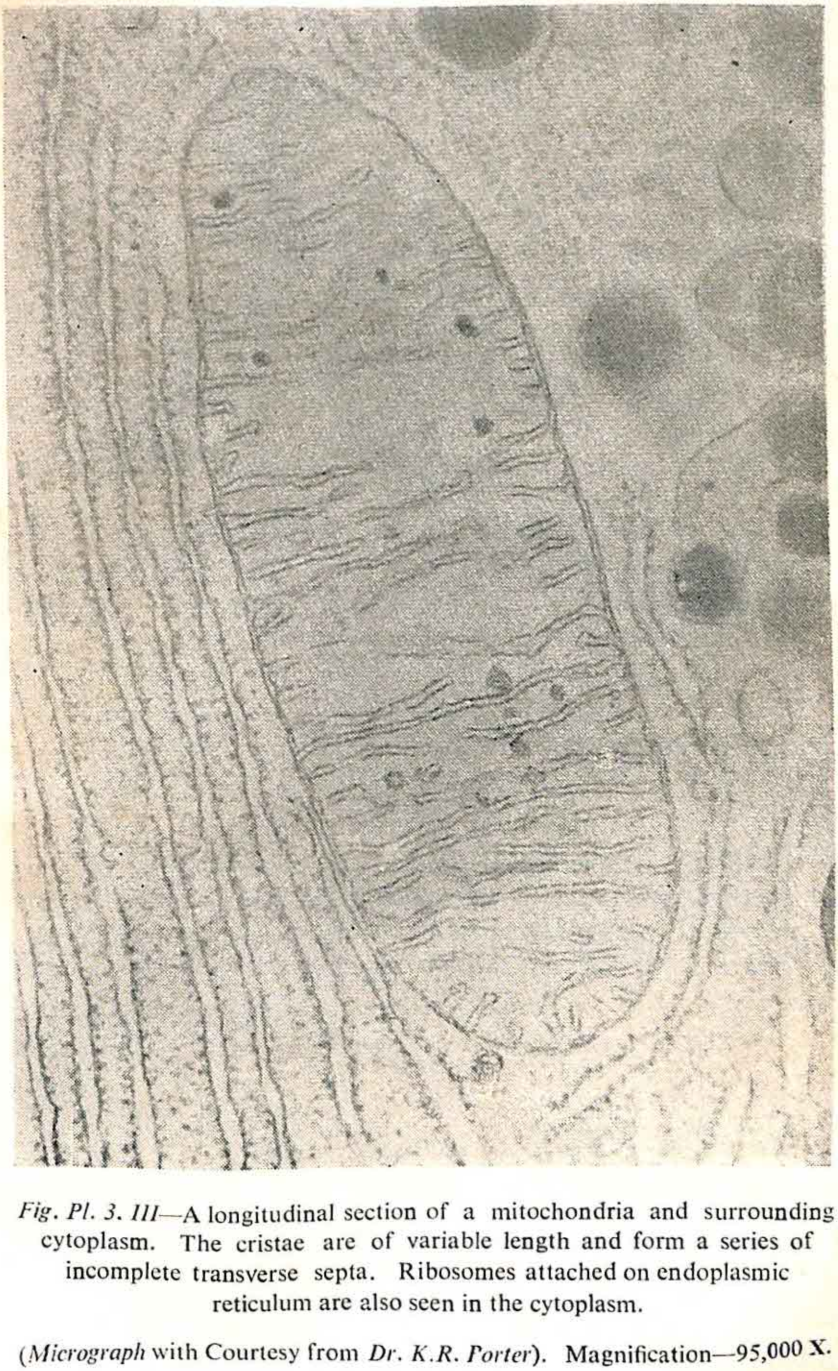


Fig. Pl. 3. III—A longitudinal section of a mitochondria and surrounding cytoplasm. The cristae are of variable length and form a series of incomplete transverse septa. Ribosomes attached on endoplasmic reticulum are also seen in the cytoplasm.

(*Micrograph* with Courtesy from *Dr. K.R. Porter*). Magnification—95,000 X.

proplastids like that of mitochondria. This fact leads us to believe the nuclear origin of plastids.

However, experiments on *Euglena* have contradicted this fact in which case plastids can arise only from the pre-existing plastids, i.e. they are autonomous structures.

(ii) *Plastids are autonomous* : This concept accommodates the fact that plastids are autonomous structures which have potentiality for changing from one to other. However, the change may be reversible or irreversible. The sequence may be from initial proplastids to leucoplasts and chloroplasts and then into chromoplasts. Leucoplasts and chloroplasts are considered as active stage of plastids while chromoplasts degenerated ones.

MITOCHONDRIA

Earlier mitochondria, as cytoplasmic particles, were known to Kalliker (1880), W. Flemming (1882) and Altman (1890) but the name mitochondria was given by C. Benda (1897) to these new structures. Mitochondria is a Greek word derived from *mitos* (means filament) and *chondros* (means granules). Since then many names have been given to this organelle, e.g. bioplasts, chondriome, chondriosome, chondriocont etc. Their presence in plants was discovered by F. Meves (1904). They are found in every living cell except procaryotic ones.

The **shape** of mitochondria is variable. Occasionally their shape is influenced by environment and physiological conditions. However, they may be granular, club-shaped, tennis-racket shaped, vesicular or rod-shaped. Like shape, the **size** also varies. The rod-shaped mito-

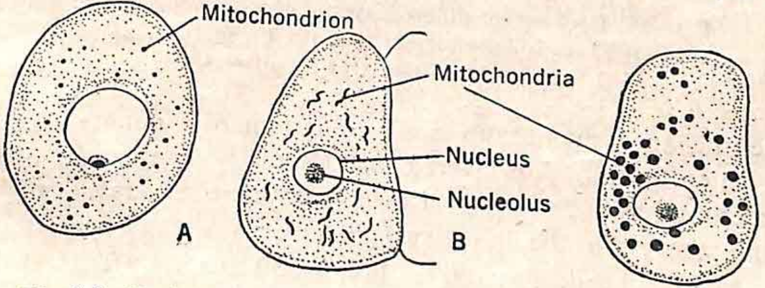


Fig. 3.5. Various sizes and shapes of mitochondria. A—in primary spermocyte of rat; B—in kidney cell of mammal and C—in liver cell of turtle.

chondria are 0·5µ to 2µ but may attain a maximum length of 7µ. The mitochondria, in general, show uniform distribution and orientation but are characteristic to species in question. The number of mitochondria per cell also varies, e.g. in the cell of rat liver, 500, 1000 and sometimes 2500 mitochondria per cell have been reported. In Sea Urchin, the number varies between 14,000 to 1,50,000 per cell.

Structure

A typical mitochondria is a sausage-shaped body of about 15,000 A° long and 5000A° in diameter. The structure cannot be seen with any kind of light microscopes and only the electron microscopy could reveal its structural organisation. Based on these studies the mitochondria have been found to be made up of two unit lipoproteinaceous membranes lying parallel to one another. The outer membrane appears to be smooth or finely sculptured but the inner membrane forms involutions inside the lumen of mitochondria which are called cristae or cristae mitochondriales. Cristae may be branched or unbranched. Cristae of animal mitochondria are pronounced and bold while plant mitochondria have delicate and finger-shaped cristae. *In* plants, the Cristae may be tubular or intermediate. On the outer surface of outer membrane and on the inner surface of inner membrane are found numerous minute structures called microbodies, (discovered by Humberto Fernandez-Moran). The microbodies of the outer surface are simple, globular, pin-head like but those on the inner surface have a stalk and bacteriophage like or rounded head. The stalk is hollow, 30 to 35A° wide and 45 to 50A° long and the head 75 to 80A° in diameter. They are spaced at roughly 100A° intervals along the membrane. These structures are also called elementary particles or inner lamellae spheres. Lehninger (1964) called them by the name oxysomes. The cavity of mitochondria

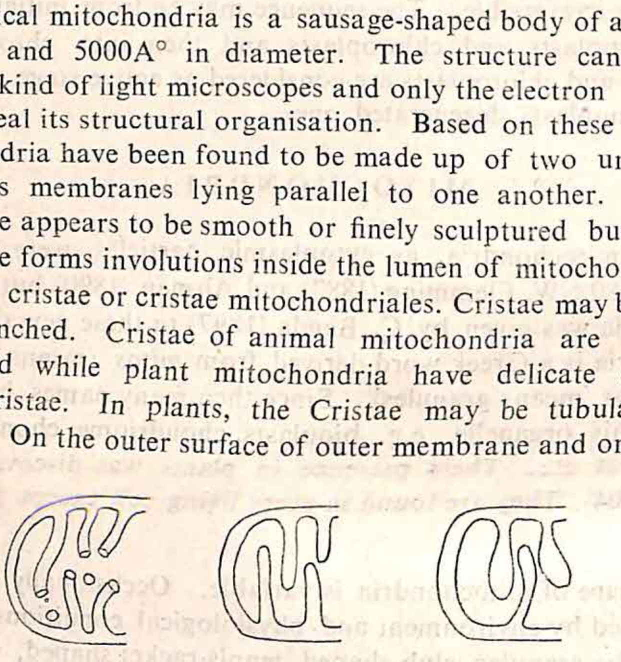


Fig. 3.6. Portions of different types of mitochondria A—tubular type (plants); B—cristae type (in animals) and C—intermediate type (in plants).

is occupied by homogenous and finely granular mitochondrial matrix but the narrow cleft between the inner and outer membrane usually appears empty.

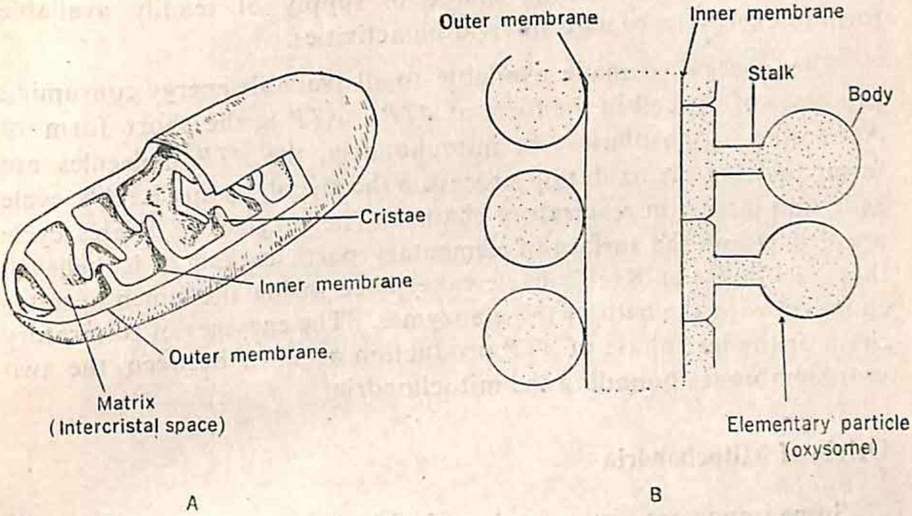


Fig. 3.7. A mitochondrion. A—a portion dissected and B—a portion of mitochondrion enlarged showing presence of elementary particles on inner membrane. These are the sites for enzymes of Kreb's cycle.

Chemically, mitochondria mainly consists of proteins and lipids constituting about 90 to 99% of the organelle. On dry weight basis its chemical composition may be as follows :

Protein—65 to 70%, Phospholipids—25 to 30%,
RNA—0·5%; *DNA*—small amount.

Enzymes constitute about 30 percent of the total protein. The presence of *DNA* has been demonstrated only in the recent years. Depending upon the size there may be one or more *DNA* molecules in single mitochondrion. In *Neurospora*, yeast, *Phaseolus vulgaris*, mitochondria contain *DNA* molecules of larger size. The mitochondrial *DNA* differs from nuclear *DNA* in basic composition, conformations (circular) and genetic complexity and also in the larger number of cytosine-guanine base pairs. It has been found that mit. *DNA* is sufficient to code for about 25 polypeptide chains but for structural proteins only. However, the protein present in cytochromes and other enzymes is dependent on nucleus. These data also suggest the presence of ribosomes in mitochondria and then a few biologists believe that mit. *DNA* represents a second genetic system in a cell.

Function

Mitochondria can well be compared with a power house of biological organisms as they are the source of supply of readily available form of energy to be used in vivid bioactivities.

The energy is made available to all available energy consuming processes of the cell in the form of *ATP*. *ATP* is the short form of Adenosine triphosphate. In mitochondria, the *ATP* molecules are formed during the oxidation process in the glycolysis and Kreb's cycle mills and largely in respiratory chains. The enzymes of Kreb's cycle are situated on the surface of elementary particles and it is believed that reactions of Kreb's cycle take place inside the lumen of mitochondria with the help of these enzymes. The enzymes of respiratory chain or the last phase of *ATP* production occur in between the two unit membranes bounding the mitochondria.

Origin of Mitochondria

Some important views are described here.

(i) AUTONOMOUS REPLICATION : This concept visualises that the mitochondria are autonomous organelles with independent hereditary action and living in the cell symbiotically. This would then represent a second genetic system in the cell. Of course, they pass on from one generation to other and in many cases their multiplication simultaneously with cell division has been observed. The concept may appeal but its universal application is doubted. However the budding of mitochondria is known in several cases. These may also arise by the formation of constriction or septum.

(ii). PROMITOCHONDRIAL ORIGIN : Mitochondria arise from smaller particles called promitochondria which are present in considerable number in meristematic cells. With the growth of the cell, the promitochondrial particles increase in volume and their inner membrane starts forming folds at right angle to the surface. Later these folds become differentiated into cristae. Some biologists believe that promitochondria may arise from nuclear envelope. Therefore, it would be more proper to say that mitochondria are of nuclear origin. Two views have been put forward to explain the mechanism of nuclear origin. Firstly, the promitochondria may arise by means of evagination from nuclear membrane forming vesicles. These vesicles grow to give rise to promitochondria. Secondly, the promitochondria

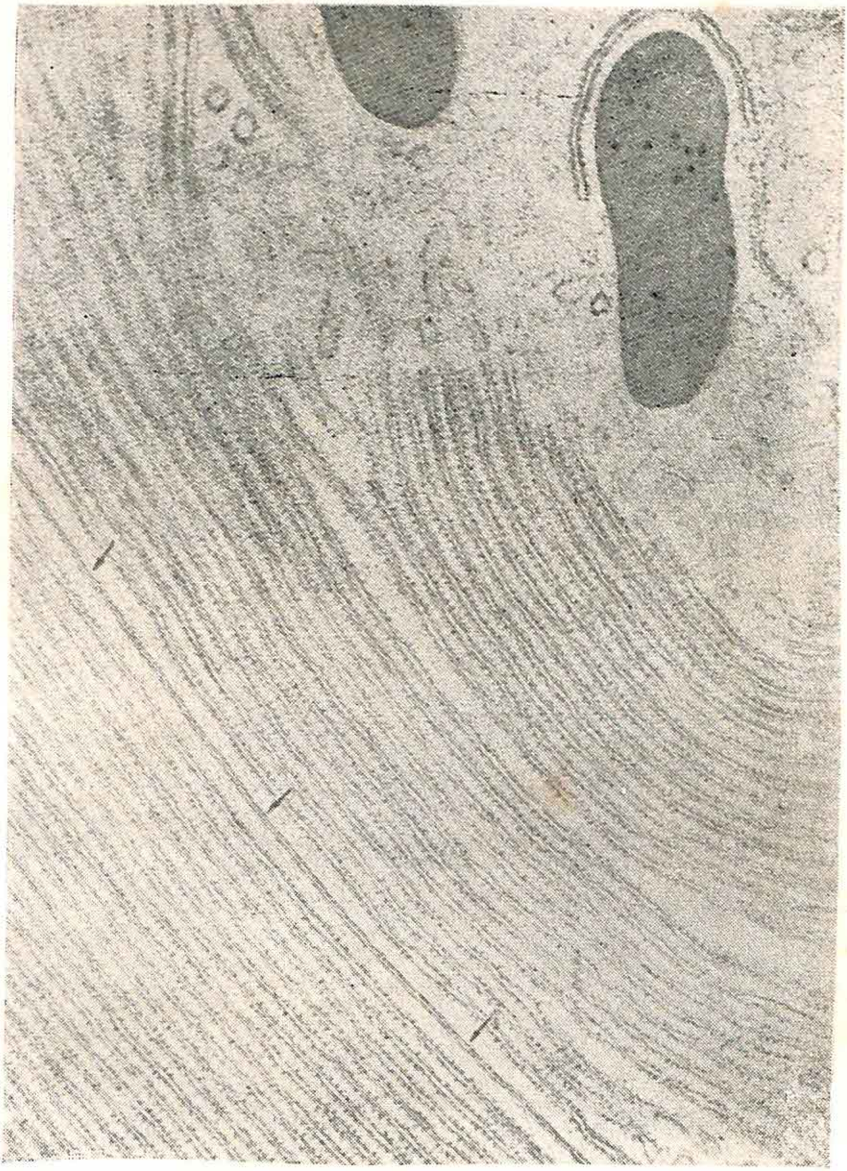


Fig. Pl. 3. IV—The cisternae (ER) at the Periphery are in close array and generally parallel to cell surface. It is in not well known what forces determine their even spacing. At right above of the fig. plate mitochondria and surrounding endoplasmic reticulum are seen. The plasma membranes of two adjacent cells cross the lower left corner (at arrows' and can be distinguished from the pairs of membranes bounding cisternae of ER, by their closure apposition and the absence of associated ribosomes.

(Micrograph with courtesy from *Dr. D. W. Fawcett*). Magnification—46,000 X.

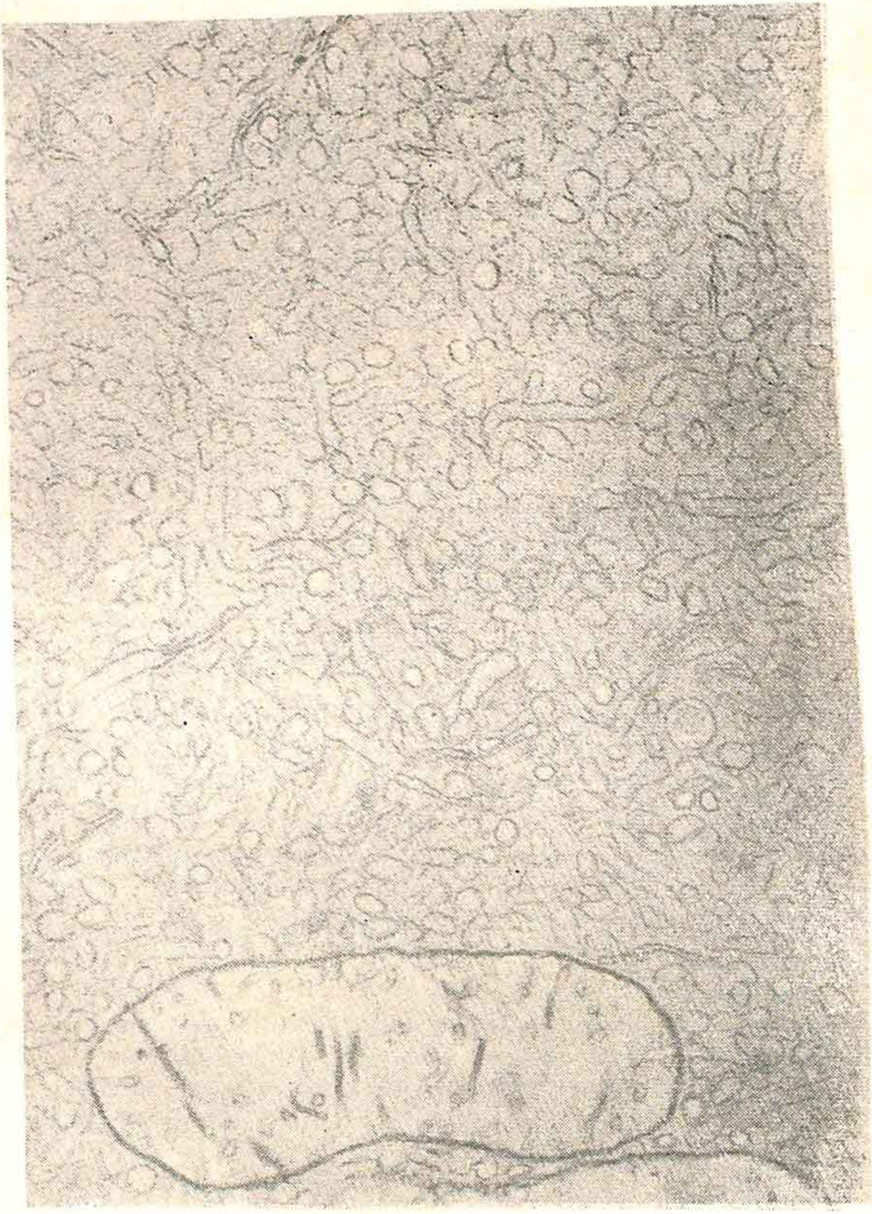


Fig. Pl. 3. V—Micrograph showing agranular reticulum which varies not only in its morphology in different types of cells, but also in thickness and stability of its membrane. The reticulum are quite thin as compared to those of neighbouring mitochondria.

(Micrograph with courtesy from *Dr. D. W. Fawcett*). Magnification—51,000 X.

may arise from the organised folding of endoplasmic reticulum. The nuclear origin of mitochondria is further supported by Armentront et al (1968). While working on *Basidiobolus* sp. and *Fusarium oxysporum* they observed the peculiar highly polymorphic mitochondria exhibiting saltatory movement which often interact with nuclei and suggest a close membranous contact between them.

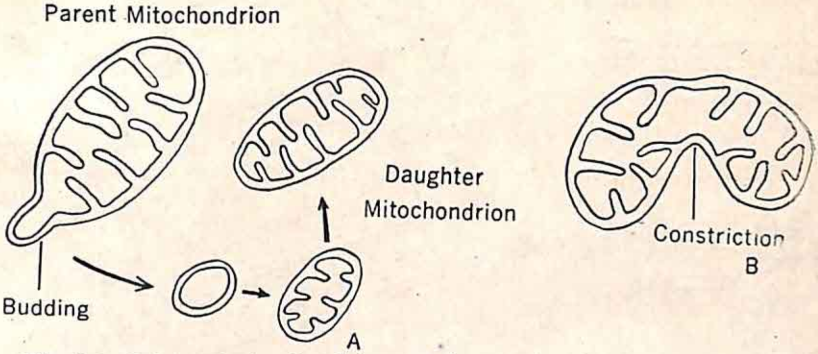


Fig. 3.8. Origin of mitochondria. A—by budding and B—by constriction.

ENDOPLASMIC RETICULUM

The endoplasmic reticulum, the name given by Porter (1955) for the organ corresponding to the basophilic components of cytoplasm, is the most fascinating discovery of electron microscopy. It is present in all living cells of plants and animals. *ER* (endoplasmic reticulum) has another interesting feature that its origin from the outer nuclear membrane has been shown and that it extends upto the cell surface. In cross-section, the whole meshwork of ER looks like organised channels but when studied with 3 *D* techniques it was found that it is composed of flat saccular expansions called cisternae. The cisternae may occur singly but more often they are aggregated to form lamellae system roughly parallel to one another. These parallel running cisternae are interconnected through partitions doing compartmentalisation in *ER*. The peripheral region of cisternae may be entire or composed of meshed network of anastomosing tubules of 400-700 A° dia. On the other end of tubules may present vesicles that in many cases do not form a continuous part of *ER* but have never been considered as separate portions. The development of tubular, cisternal and vesicular arrangement varies with the age of the cell. The *ER* may be entirely tubular or cisternal in a particular region.

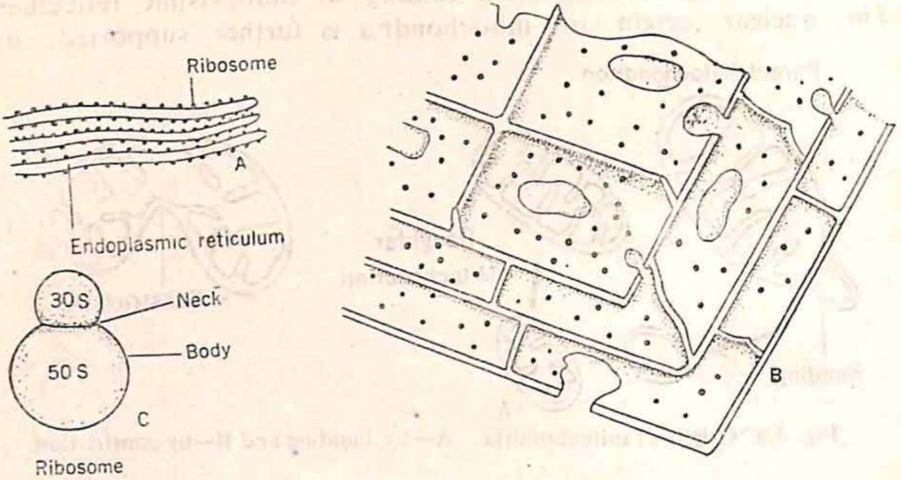


Fig. 3.9. Endoplasmic reticulum. A—a cross section of the same showing tubular appearance, B—a three dimensional diagram of *ER* showing flat structure with compartmentalisation and C—a ribosome which is found attached to *ER*.

On the basis of surface structure, two types of *ER* are known. Firstly the granular type or rough surfaced that is coated with large number of adharent ribosomes. In classical cytology, both these structures together are called ergastoplasm. Secondly, it is agranular type or smooth surfaced. While comparing the cell membrane with *ER*, the cell membranes are slightly thicker and without adharent ribosomes. The *ER* remains away about 200 A° from the general surface of cell membrane but at places in the region of plasmodesmata, it contacts and occasionally passes through to establish connections with the branches of *ER* of the neighbouring cell. Thus, the extensive mesh work of *ER* may keep the nuclei of all the cell in communication with each other directly or indirectly.

The functions of *ER* are not well understood. Besides forming the skeleton of cell, it also helps in transportation of various materials and its compartmentalisation helps in keeping certain enzymes and metabolites separate. Also the isotopic studies and electron microscopy revealed that some enzymes synthesised on the ribosomes move through the supporting membrane of the *ER* into the enclosed cavity and accumulate in the form of granules. The latter then travels through channels of *ER* to the cell surface where they are extruded..

GOLGI COMPLEX

It was discovered by Comillo Golgi (1898) in the neural tissue where it appeared as extensive network of dark strands. He called it internal reticular apparatus but other investigators referred it after his name as Golgi bodies, Golgi apparatus or Golgi complex. Occasionally, the term lipochondria has been also used for Golgi complex.

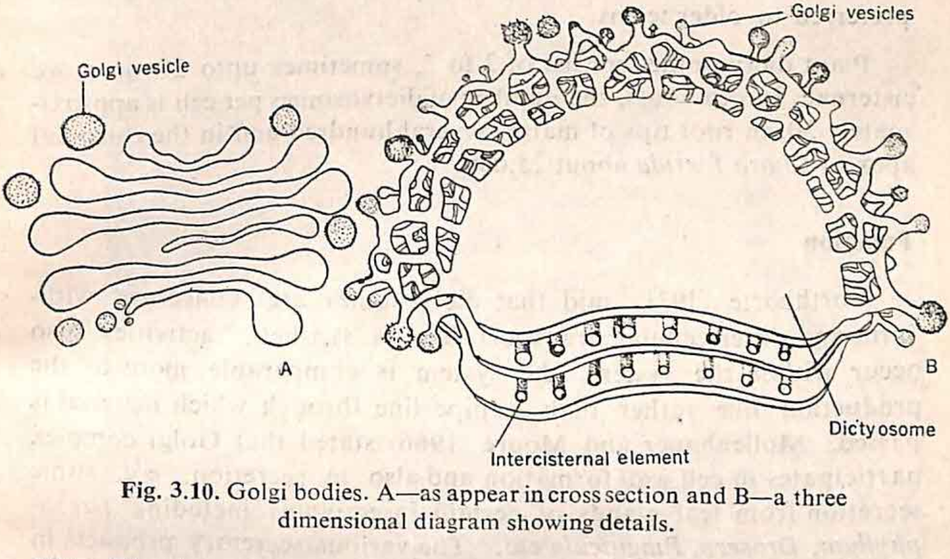


Fig. 3.10. Golgi bodies. A—as appear in cross section and B—a three dimensional diagram showing details.

Ultra Structure

Golgi complex is an assemblage of flat or curved saccules cisternae lying one above the other in close parallel array. Each cisternal unit corresponds to an individual lamella which bounds a thin closed cavity of about 150 A° across and of variable length. Four to eight such cisternal units are commonly spaced 200 to 300 A° apart and thus form multi-layered cisternae. On their convex outer surface are present several myriad small vesicles, 440-800 A° in dia. which evolve from the edges of cisternae.

The cisternae are smallest functional units separated from each other by an inter-cisternal region in a regular fashion. In this region are present several intercisternal elements composed of more or less parallel fibrous elements. The cisternae appear as fenestral plates composed of tubules which branch and rejoin to form highly fenestrated and anastomosed system. They do not appear as flattened sacs. The cisternae are so organised as to enclose several small young vesicles.

The young vesicles are smooth surfaced and older ones are rough surfaced vesicles and vacuoles. In classical cytology, the peripheral lamellar system is referred to as *dictyosomes* or *Golgi externum* and the vacuolar elements in the centre as *archoplasm* or *Golgi internum.* Because of morphological haterogeneity due to its vesicular, vacuolar and cisternal components, the term Golgi complex is now-a-days preferred to older terms.

Plant dictyosomes consist of 2 to 7, sometimes upto 20 or more cisternae. In pea roots, the number of dictyosomes per cell is approximately 30; in root tips of maize, several hundred and in the rhizoidal apex of *Chara foetida* about 25,000.

Function

Northcorte (1971) said that dictyosomes are concerned with-intra and inter-cellular transport but as synthetic activities also occur within the system, the system is comparable more to the production line rather than a pipe line through which material is passed. Mollenhauer and Moore (1966) stated that Golgi complex participates in cell wall formation and also in secretion, e.g. slime secretion from leaf glands of certain insectivores including *Drosophyllum*, *Drosera*, *Pinguicula* etc. The various secretory products in animals are Zymogen of pancreatic cells, mucous secretions, lactoprotein of mammary gland cells, compounds of thyroxin, melanin granules and other pigments etc.

RIBOSOMES

Palade (1953) for the first time reported the presence of some particulate units in the ground substance. These are small particles of about 15-200 A° dia. usually attached to the outer surface of *ER* and its vesicles. These may also be found lying free in the ground surface (cytoplasm) in actively growing cells, in bacteria and blue green algae. These are ribonucleoprotein bodies (*RNP*), i.e. nucleic acid attached to protein. Haguenau (1958) called them ribosomes.

Ribosomes are found in every type of living cell. They have been studied most in bacteria. Ribosomes found in animals and plant cells have many properties in common with those of bacteria.

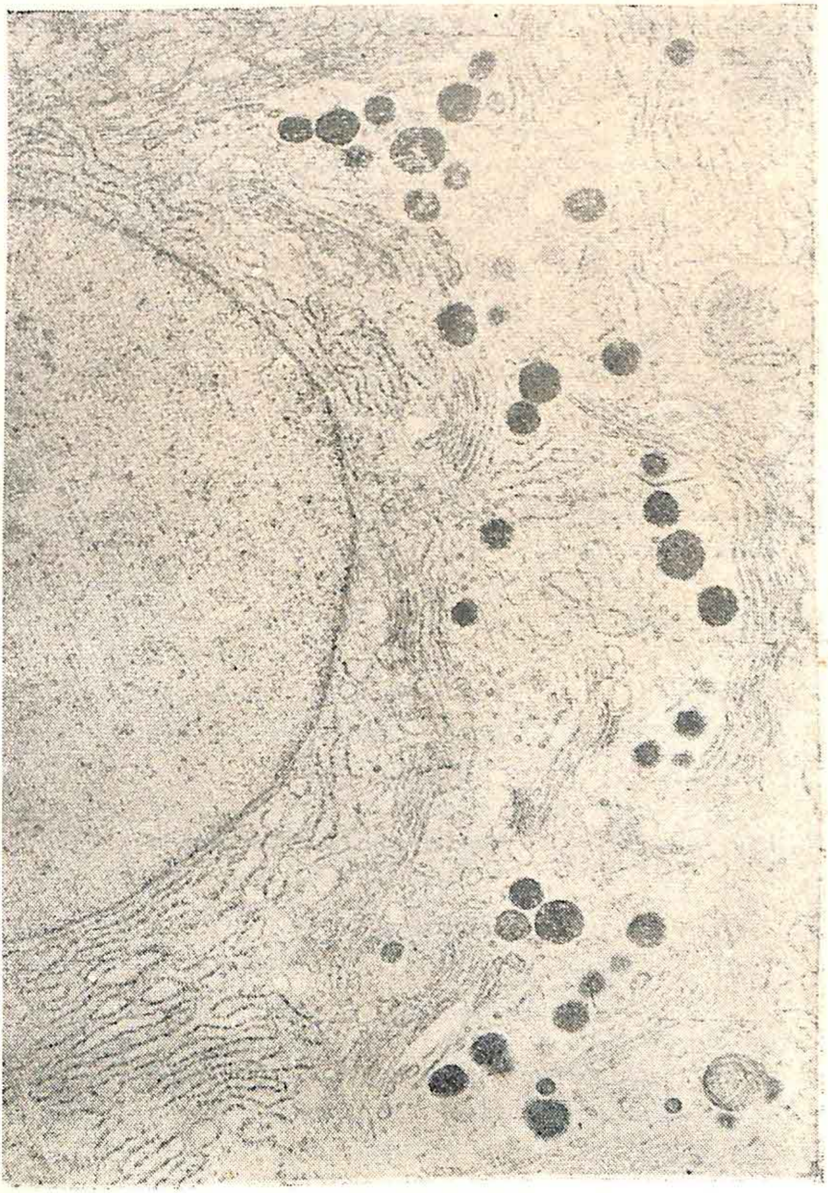


Fig. Pl. 3. VI—Micrograph showing Golgi Complex. In a more active Golgi region, the lamellar systems of cisternae are dispersed over a larger area. Secretion granules are present in greater numbers in the interior of the Golgi Complex. Nucleus is also seen on left half of the micrograph.

(Micrograph with Courtesy form *Dr. D. Friend*). Magnification — 22,000 X.

Procaryotic cells have ribosomes mainly of 70 *S* (Svedbergs unit) but these are made up of two sub-units : 50 *S* and 30 *S*, which are stapled together by magnesium ions. In exceedingly minute quantities, *Cu* and *P* may also be present. In eucaryotic cells, so far studied, ribosomes appear to be 80 *S* with two subunits—60 *S* and 40 *S*. Ribosomal *RNA* (*r RNA*) differs in size and base contents from other types of *RNA*. In *Escherichia coli*, the rate of formation *r RNA* containing at least more than one thousand nucleotides is about one molecule per second per gene. Of the total *RNA* present in an organism, 85 to 90 percent is the ribosomal *RNA*.

Peterman (1964) studied the shape of ribosomes with the help of electron microscope by shadow casting and negative staining. He observed that in *E. coli*, 30 *S* unit of ribosome is assymmetrical and is represented by a prolate ellipsoid 140 × 170 *A*° structure and 50 *S* unit as dome-shaped. The ribosomes of eucaryotic cells are comparatively spherical than those found in bacteria and are more highly hydrated. Warner et al (1962) reported that these 70 *S* particles (ribosomes) may associate into larger units, e.g. two 70 *S* particles form a 100 *S* unit. It happens especially during protein synthesis. Such association of ribosome is called polysome or polyribosome or ergosome or ribosomal cluster etc. The number of ribosomes in each polysome varies from a few to 30 or even more (Rich, 1963).

The function of ribosomes is to provide larger surface and to facilitate process of translation during protein synthesis. (For further details see chapter on Protein biosynthesis)

LYSOSOMES

One of the recent electron microscopic discoveries were the rounded dense bodies observed in the liver tissues. These bodies were described as pericanalicular dense bodies considering their location and function. These were renamed in 1955 by Christian de Duve as lysosomes on the basis of composition. These contain hydrolytic enzymes and as a matter of fact these are the lytic bodies functioning as suicidal bags.

Structurally, lysosomes are submicroscopic bodies ranging in size from 0·4 to 0·8 micron but may be upto 5 microns as in kidney cells of mammals. These are frequently found in the animal cells particularly in the cells of liver, spleen, brain and thyroid of mammals.

Their presence in plant cells is uncertain. However, in 1964. P. Matile reported the presence of lysosomes in *Neurospora*. Lysosomes show polymorphic nature.

A lysosome has a single limiting membrane composed of lipoprotein, a densely granulated stroma and a large vacuole. Chemically a lysosome is a bag filled with acid hydrolases surrounded by single unit membrane. More than a dozen hydrolytic enzymes are present in it. Some of them are Acid phosphatase, Acid ribonuclease, Acid deoxyribonuclease, Acid lipase, B-galactosidase etc. It is because of this reason the lysosome is called a body rich in hydrolases. The characteristic oxidative enzymes of mitochondria are not present in lysosomes.

Functions

Because of presence of large number of enzymes, lysosomes are capable of hydrolysing various substances. Some functions assigned to them are as follows :

(a) INTRACELLULAR DIGESTION : Lysosomes are responsible for the digestion of particles that are taken into the cell by phagocytosis. These particles when enclosed in the fragments of cell membrane are called phagosomes which fuse with lysosomes and form a single large digestion vacuole where the engulfed material is digested. Later the digestion vacuole fuses with cell membrane to expel the residual material.

(b) AUTOPHAGY : During adverse conditions, lysosomes begin to digest its own cell inclusions such as mitochondria and endoplasmic reticulum. This is called cellular autophagy which is supposed to be a process of obtaining needful energy by making use of its own constituents as a source.

(c) AGING : Lysosomes are considered as agents of aging and three different roles are assigned to them. Firstly, they produce autolytic enzymes that slowly disrupt the delicate intracellular machines. Secondly, they produce some byproducts that clog the gears of metabolic machinery and thirdly, they may digest some functional elements and then producing insoluble residues. Thus, these may be called 'suicidal bags'.

(d) AUTOLYSIS : One of the roles assigned to lysosome is the removing of dead or degenerating cells. During the process, the lysosome membrane ruptures and enzymes are set free to digest the dead cells to get rid of the cell debries. This digestive process in the dead cells is called autolysis.

(e) CELL DIVISION : Allison (1967) and others suggested that mitosis is ordinarily inhibited by some kind of repressor substance and lysosomes may be involved in this affair. It's true that dividing cells have comparatively lesser number of lysosomes that too are distributed in the peripheral region of cell.

Origin of Lysosomes

To some extent lysosomes are examples of vacuoles that appear to arise by endocytosis of the bounding membrane and also from other membranous unit within the cell. Lysosomes may exist as original lysosome or storage granule, as a phagosome or digestive vacuole, as a residual body or as an autophagic vacuole. So these may have different patterns of origin, e.g. phagosomes arise as a phagocytic vacuole and residual body as remanant of phagosome.

TRANSOSOMES

Known so far only in the avian ovarian follicle, these independent organelles called transosomes were discovered by Press (1964). In general, these organelles are spheroids consisting of triple bonded envelope with several ribosome-like granules attached by short stalks to the innermost band. Hardly any exact chemical composition is known but they are composed of *RNA* and may have some types of enzymes. These are formed at the surface membranes of follicular cells. It is believed that they have some role with the developing yolk granules, but their exact function is not known.

GLYOXYSOMES

Discovered by Beevers, glyoxysomes are spherical particles of 0·5-1.0 μm. These were observed in a crude mitochondrial preparation from castor bean endosperm. These have single unit membrane enclosing a finely granular stroma and are concerned with glyoxylate cycle. These contain isocitrate lysase and malate synthetase. However, these are known only from the tissues where fat is converted into carbohydrate.

MICROTUBULES

Microtubules are known only from plant cells and are restricted in the peripheral cytoplasm immediately within the cell membrane.

Such microtubules have also been seen within the mitotic spindles. Several microns long microtubules are nearly 240 A° in diameter and are hollow.

Their nature of function is not perfectly known. However, these are related to execute the orientation of the newly synthesised cellulose microfibrils. These may also help Golgi bodies to move to come in contact with cell membrane. It is speculated that their role is also connected with cytoplasmic streaming and intracellular transport.

PEROXISOMES

Concerned with glycolate metabolism and photorespiration and discovered in the spinach leaves, these microbodies were found closely associated with the chloroplast and were discovered by Tolbert and his co-workers (1968). Since then they have been reported occurring frequently in plants that show photorespiration and are responsible for the metabolism of glycolic acid. A close relationship between chloroplast and peroxisome has been suggested and Frederick and Newcomb (1969) believe that some exchange of material occurs in between two. The detailed structure of peroxisomes is yet to establish but these definitely contain enzymes for glycolate metabolism. Peroxisomes are known from the plants particularly in spinach, sunflower, tobacco, pea and wheat.

SPHEROSOMES

Spherosomes are minute spherical bodies lying free in the cytoplasm and thought to be involved in fat synthesis. Bounded by single unit membrane these are probably formed by the pinching off of small bits of endoplasmic reticulum. These may be involved in certain other activities too. Sorokin (1967) found the presence of mostly phospholipids and very little neutral lipids and reserve oil droplets of similar nature while Holcomb et al (1967) observed the presence of hydrolytic enzymes in these organelles.

CENTROSOMES

Discovered by T. Boveri (1888), the centrosome is the circumscribed region of clear cytoplasm adjacent to the interphase nucleus from which radiate spindle fibres as an aster during cell division in the animal cells.

Each centrosome consists of two cylindrical centrioles of about 3000 A°-5000 A° in length and 1500 A°-1800 A° in dia. located at right angle to each other. Electron microscopy has revealed that centriole consists of 9 triplets arranged around a central axis. Each triplet fibre consists of three secondary fibres each about 200 A° in dia. The triplet fibres are arranged in a line tilted at about 40° to the tangent of centriole. Besides, the appendages known as satelite also extend radially from a group of centriole fibres. The whole structure is enclosed in a thick membrane and the cytoplasm around the centriole is called centrosphere. Both centriole and centrosphere together form the centrosome. Besides proteins that form fibres, some amount of *DNA* present in centrosome has been also reported.

Centrosomes are found in animal cells but also in certain lower plants such as *Chlamydomonas* and *Spirogyra.* The centriole play a significant role in cell division which is discussed in separate chapter. About the origin, the centrosomes are semi-autonomous.

CILIA AND FLAGELLA

Cilia and flagella are delicate cytoplasmic extensions of the cell surface. These are contractile filaments of variable size and number. Internally, both cilia and flagella do not differ from each other. However, flagella are longer and cylindrical and fewer in number and each flagellum shows independent movement while cilia are shorter, little flat and are more in number. Cilia exhibit a combined action during movement. Both are common to plant and animals.

Electron microscopic studies revealed that each cilium remains surrounded by a membrane arising from plasma membrane. There are about 9 pairs of fibrils, each about 80 A°, arranged in a sphere in the peripheral region. Of the pair one

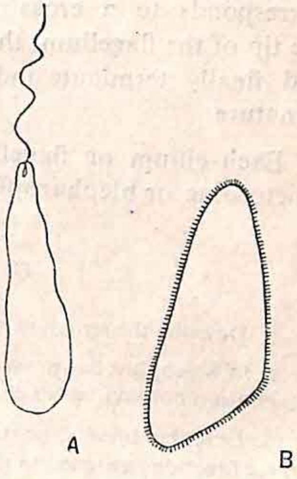


Fig. 3.11. A—cell showing presence of flagellum and B—presence of cilia all around the surface.

is called subfibril A that occasionally possesses arms—50 A° thick and 150 A° long and the other subfibril B. The centre is occupied by a pair of central fibre. Besides, nine dots in the area in between central fibrils and sub-fibrils are also present called secondary fibrils. Each corresponds to a cross section of fine longitudinal filaments. At the tip of the flagellum, the outer fibres converge and become single and finally terminate independently. The fibres are proteinaceous in nature.

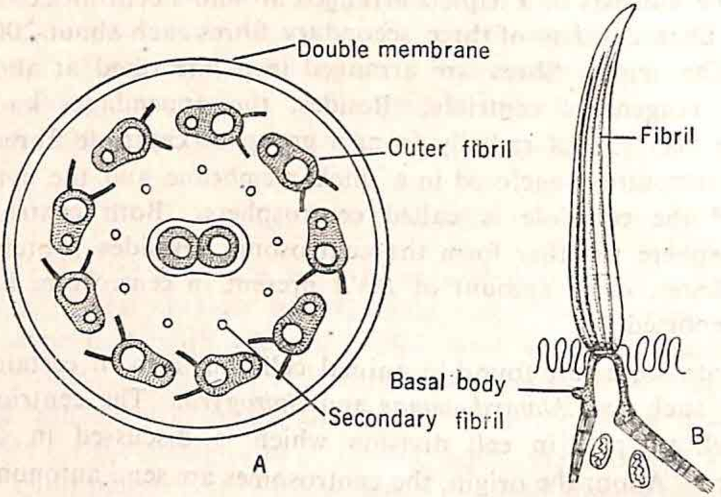


Fig. 3.12. A—C. S. of flagellum and B—details of flagellum.

Each cilium or flagellum arises from basal body (also called kinetosome or blepharoplast) which in turn gives rise to rootlets.

GENERAL QUESTIONS

1. Describe the structure and composition of chloroplast.

2. "Chloroplast has proved as most interesting organelle of the cell and its orgainsation has seen many changing faces." Comment.

3. Describe briefly the ultrastructure of mitochondria and mention the various functions assigned to it.

4. Give an account of structure and function of Endoplasmic reticulum.

5. While discussing the ultrastructure of Golgi complex (Golgi apparatus) mention critically the functions and origin of it.

6. What are ribosomes and polyribosomes ? Give briefly the structure and composition of these structures.

7. "Lysosomes are called suicidal bags." Discuss about the statement and cover briefly its structure and functions.

8. Write short notes on—(i) Quantasomes, (ii) Oxysomes, (iii) Dictyosomes, (iv) Transosomes, (v) Glyoxysomes, (vi) Microtubules, (vii) Peroxisomes, (viii) Spherosomes, (ix) Centrosomes, (x) Cilia and flagella.

■ ■ ■

CHAPTER 4

NUCLEUS AND THE CHROMOSOMES

THE NUCLEUS

The nucleus as a prominent body within a cell was first discovered by Robert Brown in 1831. Since then its role in heredity, cell divisions and cell metabolism has been variously studied but the first empirical demonstration of its significance was experimentally shown by German biologist J. Hammerling in 1934.

Hammerling's Experiment

Hammerling proposed that the nucleus determines the characters of cell and ultimately the characters of the individual. For the demonstration of its role, he selected two different species of a unicellular green alga : *Acetabularia—A. crenulata* and *A. mediterranea,*

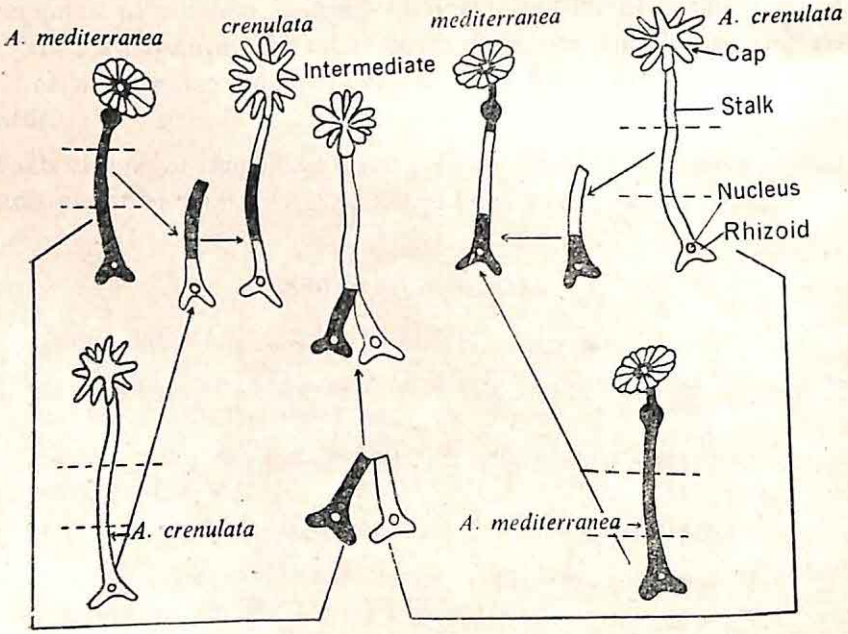


Fig. 4.1. Hammerling's experiment in *Acetabularia* showing the roles of nucleus and cytoplasm.

both showing differences in the morphology of their respective caps. In *A. crenulata*, the cap has loose rays and in *A. mediterranea* an umbrella-like cap is found. The nucleus in these plants lies in their rhizoidal regions. In each case, he observed that if the cap is removed, another similar cap will develop from its rhizoidal region.

So in his experiment, after removing the cap, he grafted the stalk (without nucleus) of one species on the rhizoidal portion with nucleus of other species and found that the shape of the cap is determined by the nucleus and not by the cytoplasm of the stalk. That is to say if the nucleus in a graft comes from *A. crenulata*, the shape of the cap will be crenulata type. It was also found true for reciprocal grafts. To ascertain the immediate effect of respective nuclei he manipulated the grafting so as to bring two nuclei in one graft hybrid, and found that the shape of the cap being formed intermediate type.

Thus, the experiments on *Acetabularia* provide convincing evidences to establish the fact that the expression of characters of an individual is controlled by the nucleus of the cell.

Structure and composition of nucleus

Surrounded by the cytoplasm and normally occupying about two third of the cell space is found the spherical nucleus that controls all the activities of the cell. Normally a cell contains a single nucleus but the occurrence of multinucleate cells is also there in several cases, e.g. in *Cladophora*, *Pithophora*, *Hydrodictyon*, laticiferous cells in angiosperms etc.

A typical nucleus is composed of a few prominent structures such as *nuclear membrane*, *nuclear sap*, *nuclear ribosomes*, *nucleolus* and *chromatin network*.

The nucleus remains externally bounded by lipoproteinaceous two unit membranes that run parallel to one another with a gap of about 200 to 400 A°. This gap is called perinuclear space which is in fact an electron transparent area. Electron microscopic studies have revealed that the boundary between the nucleus and the cytoplasm has a

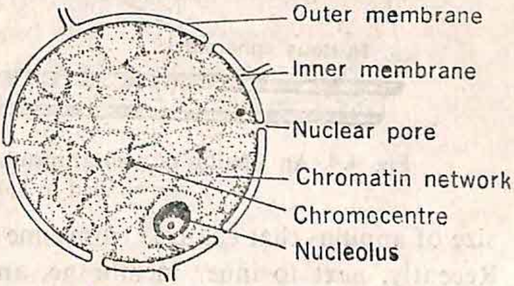


Fig. 4.2. An interphase nucleus.

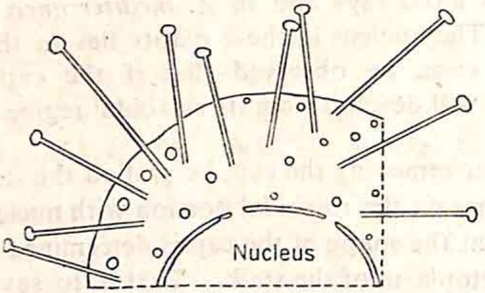


Fig. 4.3. A part of nucleus showing the presence of microtubules on the surface.

complex double membrane structure. Considering this fact, the term **nuclear envelope** is now preferred to the term nuclear membrane. The outer membrane at places gives out tubular structure commonly referred as endoplasmic reticulum. In certain cases, microtubules on the surface of nuclear membrane are also present. Besides, the nuclear envelope possesses here and there openings of 300-600 A° size, called annuli, through which nucleoplasm communicates with cytoplasm. These annuli (*singular*—annulus) can not be called as pores as they do not represent permanent openings in the nuclear envelope. Yoo and Bayley (1967) observed eight spheroid particles arranged around the circumference of each annulus in the nuclear envelope of pea seedlings. Perhaps these regulate the size of annulus that changes from time to time according to the need. Recently, next to inner membrane, an *internal dense lamella* region of 200 to 800 A° size has been recognised. It is considered as a more or less constant structural element of the nuclear envelope. The inner side of lamella is generally bordered by elements of chromatin network.

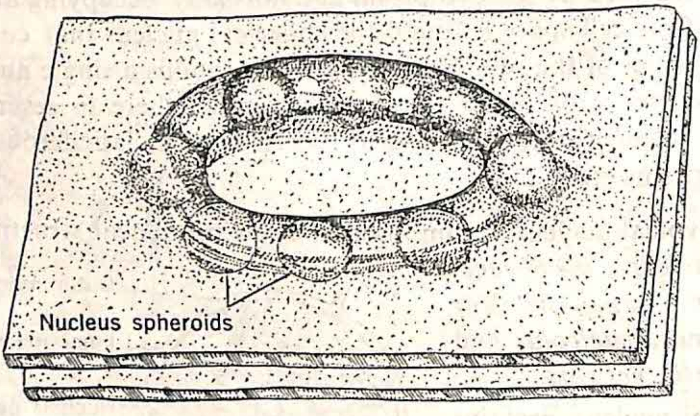


Fig. 4.4. An annulus of nuclear membrane enlarged. It remains surrounded by spheroids.

Encircled by the nuclear envelope is found the **nuclear sap** (nucleoplasm or karyolymph). The nucleoplasm is a semi-fluid substance which contains nucleic acids, nucleoproteins (both histones and protamines) etc. and forms the matrix. **Nucleolus** (*plural*—nucleoli) is found lying in the nucleoplasm. There may be one nucleolus or more than one nucleoli in each nucleus. It depends upon the species in question. These nucleoli are spherical bodies made up of small granules containing proteins and *RNA*. No separate membrane has been detected for the nucleolus. The recent studies have revealed that a typical nucleolus exhibits two distinct structural regions. The core is the central region being composed of compact fibrous elements. It remains surrounded by granular area called cortex. The two regions may be intermixed in certain cases. It is believed that nucleoli help in the synthesis of nucleoproteins. Bonner (1965) while working on pea embryos has shown that ribosomes have a nuclear origin and in this origin nucleolus appears to play a key role.

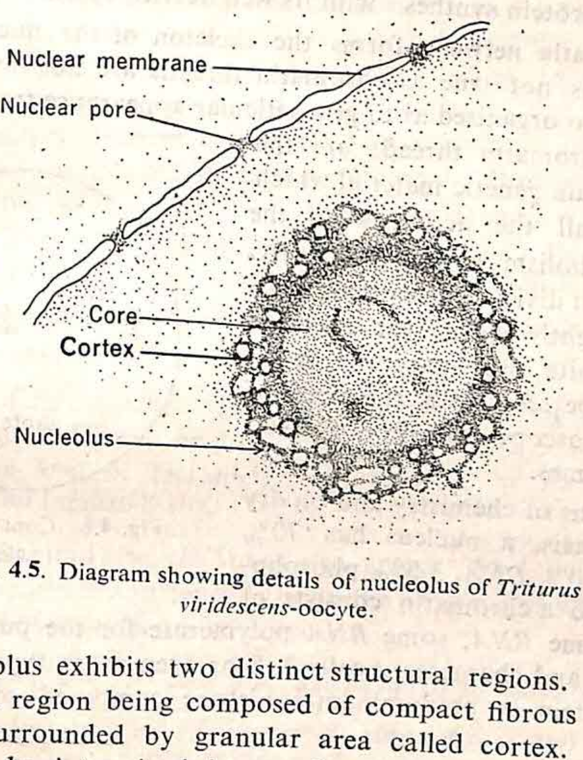


Fig. 4.5. Diagram showing details of nucleolus of *Triturus viridescens*-oocyte.

The **nuclear ribosomes** were first shown by Ts'o and Sato (1959) who on the basis of chemical and physical characterisation stated that they are identical to cytoplasmic ribosomes. In the process of formation, *RNA* fragments are first taken by nucleolus and are then transferred into ribosome incoroporated with protein. Thus nuclear ribosomes are those ribosomes whose *RNA* has been most recently synthesised. Rho and Bonner (1961) have shown that *RNA* which appears in cytoplasmic ribosomes passes through the nucleolus in its journey from chromatin where it is generated to its ribosomal destination, i.e. nucleolus receives *RNA* from chromatin and clothes it in ribosomal protein. Bonner (1965) reported that in pea embryos, the ribosomes are closely bound in nucleolus just as in the cytoplasm they are bound to the endoplasmic reticulum. It reveals that nucleolus is relatively inert in *RNA* synthesis and is exceedingly active in protein synthesis with its well devised system.

Chromatin network forms the skeleton of the nucleus. Infact, network is not true as chromatin threads are not interconnected. They are so organised as to give reticular appearance from the surface view. Chromatin threads are the sites of main genetic material which controls all the activities of the cell, metabolism as well as heredity. During cell division, the chromatids become tightly coiled, get enclosed in a definite proteinaceous matrix and can be seen with the help of light microscope. These are called chromosomes.

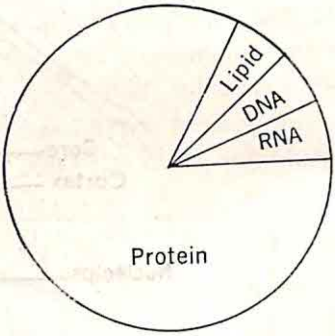


Fig. 4.6. Constituents of nucleus.

In terms of chemistry and on dry weight basis, a nucleus has 70% protein, 10% *DNA*, 3-5% phospholipids and a chromatin consists of *DNA*, some *RNA*, some *RNA* polymerase for the purpose of *RNA* synthesis and characteristically 3, 5 or even more types of proteins. This protein is predominantly histone composed of basic amino groups.

THE CHROMOSOMES

The thread-like structures which appear during cell division were discovered by Strasburger (1875). At that time he was never knowing that these structures called chromosomes (Greek word; *chroma* = colour and *soma* = body) will become the most significant component of the cell. Chromosomes are now known present in the highly organised fashion within the nucleus and are specific to their number, size and shape to every nucleus. In interphase of nucleus, they appear as deeply staining reticulum which start contracting while preparing for mitosis or meiosis. These highly contracted structures are visible during the cell division under the compound microscopes.

Morphology of Chromosomes

The morphological aspects of a chromosome are studied during metaphase or anaphase. Some important points are discussed here.

The number of chromosomes in a nucleus varies to a great range. In *Haplopappus gracilis* (a member of Compositae), a diploid nucleus contains only 4 chromosomes while in some pteridophytes there may be more than 1200 chromosomes in a diploid nucleus. In *Aulacantha*, some 1600 chromosomes are found in each nucleus. The number of chromosomes per nucleus is definite and characteristic to every individual and are thus helpful in ascertaining the phylogenetic status and taxonomic position of an individual. Haploid chromosome number in *Drosophila melanogaster* is 4, in man 23, *Pisum sativum* 7, *Allium cepa* 8 etc. A haploid set of chromosome which is inherited is called genome and the whole collection of chromosome in a nucleus is referred as chromosome complement.

The size of chromosome also varies. These may be as short as 0.25μ in fungi and birds or as long as 30μ in some plants such as *Trillium*. The average metaphase chromosome of *Drosophila* is 3.5μ, man 5μ and maize 8-10μ.

Like number and size, the shape also varies. The shape is often observed at anaphase and depends upon the position of centromere. Accordingly these may be of four types :—

(i) TELOCENTRIC—The centromere is at the tip of the chromosome.

(ii) ACROCENTRIC—The centromere is subterminal and therefore chromosomes appear as rod-like having a very small arm and other very long.

(iii) SUB-METACENTRIC—The centromere is not exactly in the centre but is close to it and therefore the chromosome has unequal arms resembling j-shape.

(iv) METACENTRIC--Centromere is situated in the centre or middle part of the chromosome and therefore it has two equal arms. Normally, it appears *V*-shaped.

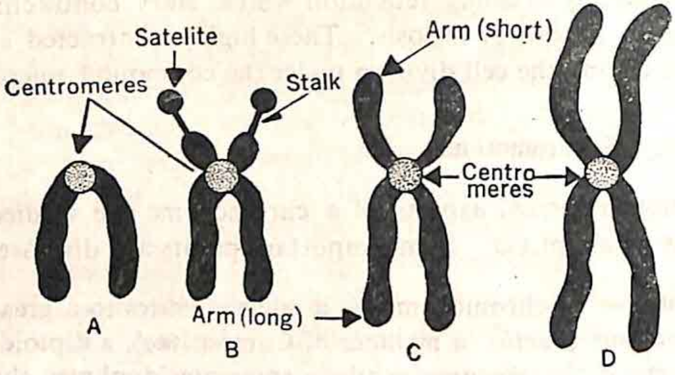


Fig. 4.7. Chromosome morphology. A—telocentric, B—acrocentric, C—submetacentric and D—metacentric.

Structure of Chromosome

Chromosome is the most wonderful gift of the nature. History of genetics so far goes after it to unravel its mystery. Despite extensive researches on the structure of chromosome there remains much to study.

However, chromosomes in general have some common features. Each chromosome has a constriction, almost centrally located, called centromere or primary constriction which executes two important functions :—

(1) To have mechanism to hold two sister chromatids together until the end of metaphase.

(2) To maintain site for the attachment of spindle fibres in order to transport the sister chromatids to opposite poles.

Electron microscopy has revealed that these two functions are assigned to two separate structures. It is the chromatin fibres of the centromere region that act to hold the sister chromatids together while the attachment of each chromatid to spindle is mediated by special dense structure called kinetochore. Kinetochores are coin-shaped in many mammals and ball-shaped in plants. However, very little is known about the chemical aspects of kinetochore and about the mechanism that brings about attachment of spindle fibres at the site (Bajer, 1970).

Centromere is often used as a point of classification and accordingly a chromosome may be telocentric, acrocentric, submetacentric or metacentric (described earlier).

Chromosomes divide lengthwise into two halves during cell division. Each part is called chromatid. The two lengthwise organised chromatids remain held together at a centromere. These chromatids when stretched, particularly as in lamp-brush chromosome, are found composed of many threads called chromonemata. Chromonemata were discovered by Vejdovsky (1912). On these chromonomata are present several bead-like areas of increased density dispersed along them, the chromomeres. These chromomeres appear as bands in salivary gland giant chromosome of *Drosophila*.

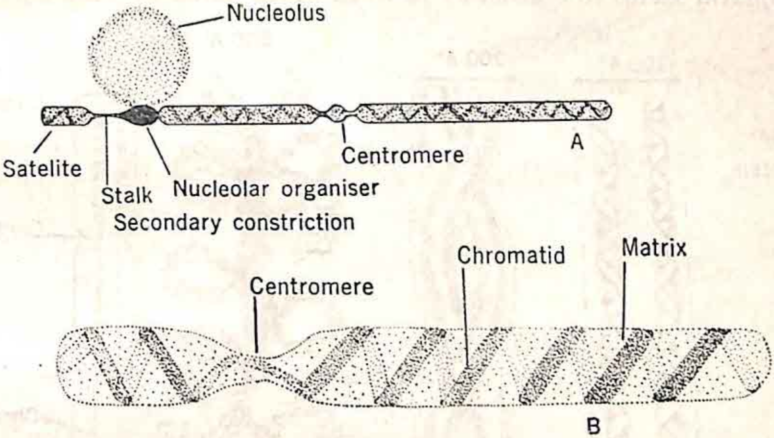


Fig. 4.8. Morphology of chromosome.

Besides primary constriction, the secondary constrictions in one or both arms of a chromosome may also be present. Usually only one secondary constriction is present in each chromosome. The small segment of the chromosome distal to this constriction is called satellite. In certain cases, secondary constrictions are seen intimately associated with the nucleolar organiser.

Chemistry of Chromosomes

The chief constituents of a chromosome are *DNA*, *RNA* and proteins. Proteins are both histone and non-histone types. Calcium is also present in addition to these components. The chemistry of *DNA* and *RNA* is discussed elsewhere.

Fine Structure of a Chromosome

Investigations on ultrastructure of chromosome is extremely difficult because of great complexity and both circumstantial evidences and hypotheses have been visualised in establishing it. Even electron microscopy has failed in this respect. The complexity in structure is largely due to coiling and supercoiling of the elementary fibrils of which a chromosome is composed of. However, the lamp-brush chromosomes are largely uncoiled. Ris (1960, '61) has shown that the fundamental unit of a chromosome is a fibril of 100 A° dia. It is called elementary chromosome fibril. Such fibrils consist of two 40 A° fibrils which correspond to *DNA*-histone molecules. He suggested that in most chromosomes, the individual chromatid seems to consist of 16 to 32 fibrils lined up side by side.

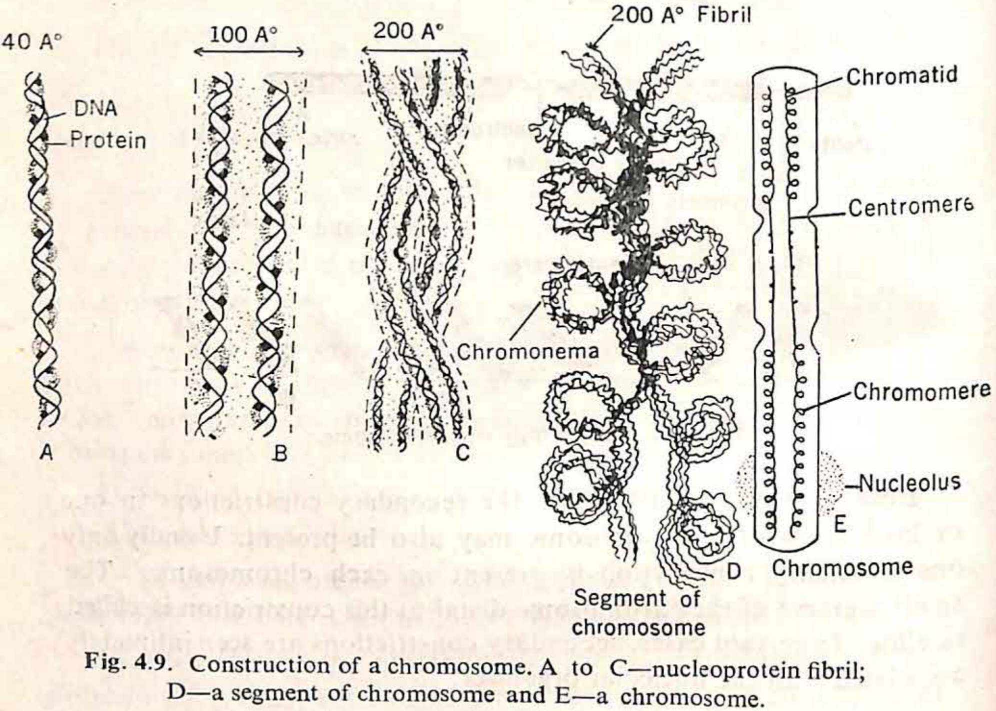


Fig. 4.9. Construction of a chromosome. A to C—nucleoprotein fibril; D—a segment of chromosome and E—a chromosome.

So in general, the elementary chromosome fibril probably consists of two *DNA*-histone molecules arranged side by side and possibly cross-linked through histones or other proteins. These fibrils run lengthwise of the chromatid and may be joined end to end by non-histone protein. Such fibrils are replicated many times in a chromatid and are randomly coiled. The whole mass of the chromatid is coiled again. When studied the lampbrush chromosome, it has been found that *DNA* strand, which runs continuously along the chromonema, takes part in some complex coilng in the centromere, then extends out into each side loop and returns to join the chromomere before continuing again in the chromonema (Paul, 1969).

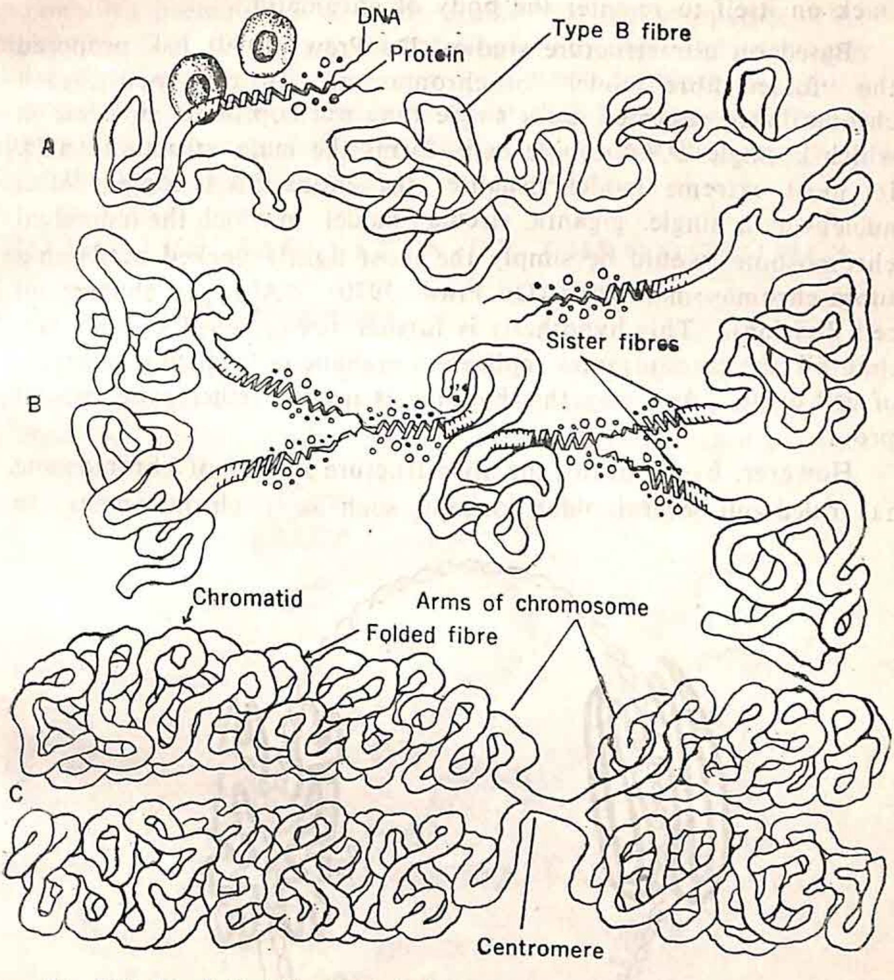


Fig. 4.10. The folded fibre model of chromosome. A—unit chromatid; B—showing simultaneous replication of chromosome at several sites; C—The late replicating segments of the fibre at the centromere which serves to hold together the sister chromatids (Redrawn—Du Praw, 1970).

The folding and packing patterns within a chromosome are thought to vary in specific ways related to genetic functions. The earlier concept that 2, 4, 8 or even 16 identical *DNA* units run parallel which are supposed to terminate with free fibre ends at the opposite of telomeres of the chromatids has not been observed by whole mount electron microscopy. Instead, there are numerous loops in which a chromatin fibre emerges out and then doubles back on itself to re enter the body of chromatid.

Based on ultrastructure studies, Du Praw (1970) has proposed the "folded fibre model" of chromosome. In this model, each chromatin is regarded as a single long nucleoprotein molecule in which a single *DNA* double helix forms the main structural axis. Its most extreme model visualises the entire *DNA* content of a nucleus as a single, gigantic circular model, in which the individual chromosomes would be simply the most tightly packed beads on a supra-chromosomal string (Du Praw, 1970). (Also see chapter on cell division). This hypothesis is further strengthened by the fact that all the chromosomes replicate simultaneously during *S* period of cell cycle. Any way this hypothesis needs further experimental proofs.

However, by all means, the ultrastructure studies of chromosome has ruled out several older concepts such as (i) chromosomes are

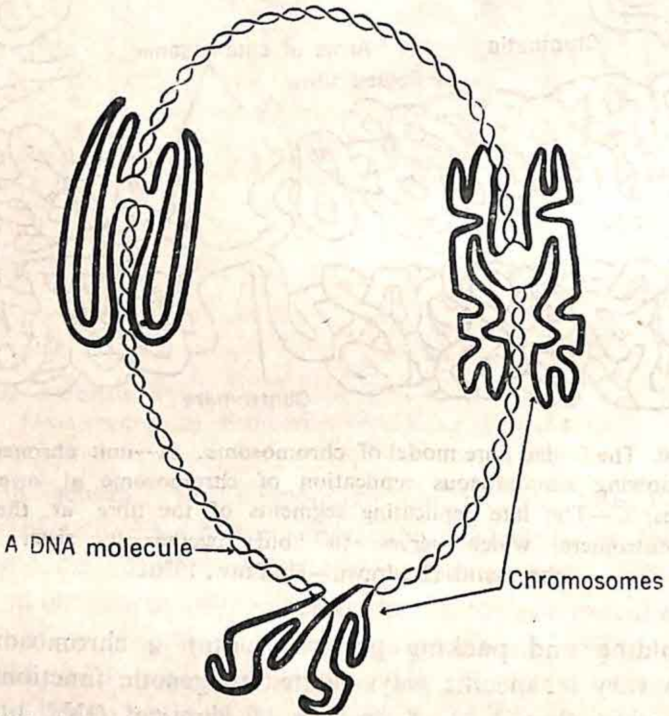


Fig. 4.11. Diag. to show that all the chromosomes of a nucleus are connected as part of a single circular and enormously long *DNA* molecule.

individually enclosed in a membrane, (ii) there would be a separate *DNA* molecule for each gene and (iii) each chromatid would have to contain a protein core to hold together the many separate *DNA* molecules. To the larger extent it is now believed that each chromatid corresponds to one very long *DNA* double helix. *DNA* is first tightly packed by repeated super coiling into a shorter thicker fibre which is then folded both longitudinally and transversely to form the body of the chromatid.

SOME SPECIAL TYPES OF CHROMOSOMES

1. Salivary Gland Chromosomes

Balbiani (1881) for the first time observed some giant chromosomes as coarse strands in the cells of salivary glands of the *Chironomus* larva. Koller named them polytene chromosomes because they appear to be formed of several chromonemata which may vary from 512 to several thousands (16,000 chromonemata). On staining, the polytene chromosome shows dark characteristic bands of varying widths which are composed of chromomeres of individual chromonemata in a linear array and perpendicular to

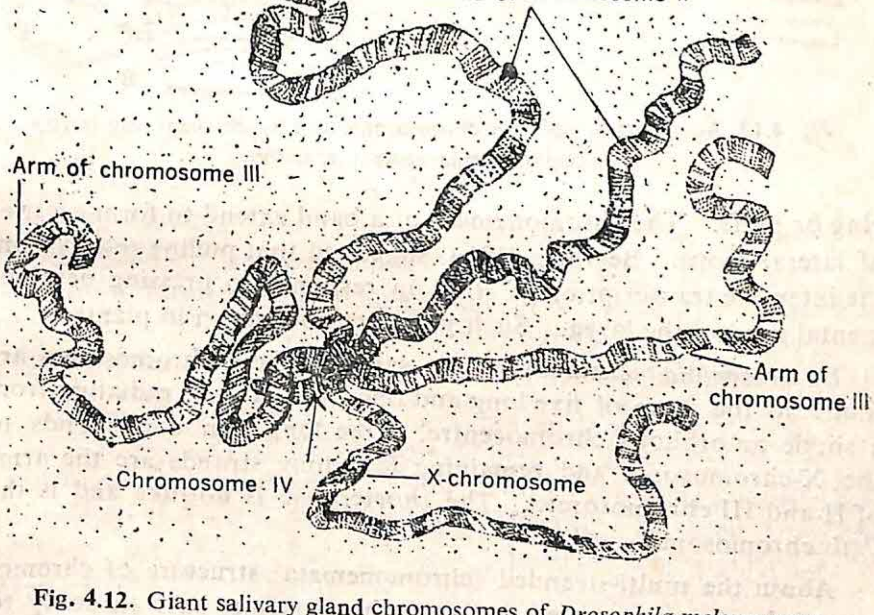


Fig. 4.12. Giant salivary gland chromosomes of *Drosophila melanogaster*.

the axis of the chromosome. The bands are rich in *DNA* but also contain *RNA* and some basic proteins whereas interband region contain less *DNA* together with acid protein. Though a band does not represent to a single gene yet they are of specific value in genetic studies. There are suggestive evidences to hold that interbands are also genetically active.

At certain stages of larval development, specific band of polytene chromosome shows enlargement in the form of bulbs called Balbiani ring or puffs. The chromonemata of a band extend to form a series of lateral loops. Beerman (1964) suggested that puffing results from the intensive transcription of *RNA* in response to pressing developmental need of the larva. Such puffs are not known in plants.

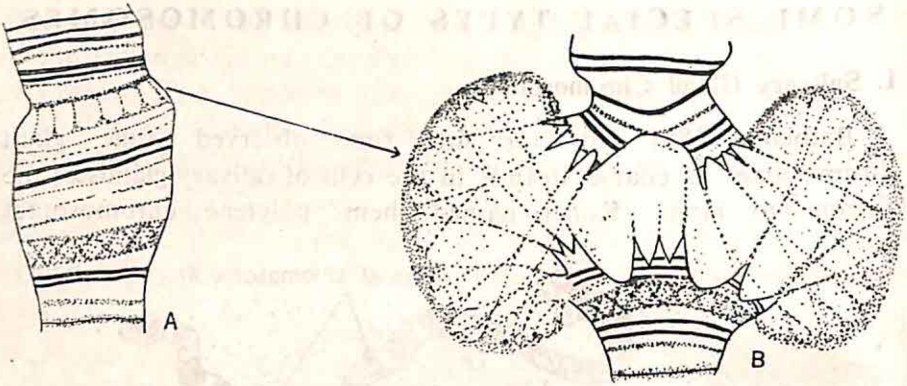


Fig 4.13. A—a part of polytene chromosome and B—Balbiani ring (arrow shows region of enlargement or puffing).

In *Drosophila melanogaster*, the salivary gland chromosomes are found in the form of five long and one short strands radiating from a single amorphous chromocentre. One long arm corresponds to the X-chromosome and remaining four long strands are the arms of II and III chromosomes. The shorter one is dot-like and is the IV*th* chromosome.

About the multi-stranded (chromonemata) structure of chromosome it has been suggested that such chromosomes have tendency to replicate during cell division but they do not separate and remain linked together and thus result in extra-thick or giant chromosomes. These chromosomes are specially suitable for genetical studies.

2. Lamp brush chromosomes

These are special types of chromosomes found in variety of primary oocytes of sharks, amphibians, reptiles, birds etc. In *Drosophila*, these are found during prolonged diplotene stage and are

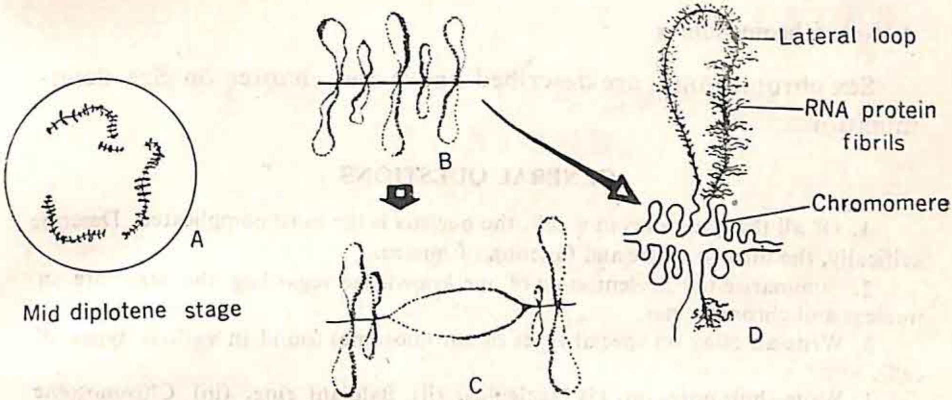


Fig. 4.14. Lamp brush chromosome. A—as appear during mid-diplotene stage; B—a portion enlarged; C—a segment is shown stretched and D—an enlarged lateral loop of lamp brush chromosome.

characterised by a remarkable change in structure and enormous increase in length. The largest chromosome reaching upto 1 mm. length have been observed in Urodele amphibian. Thus, these may be quite large as to be seen by naked eyes.

These have fine lateral loops projecting in pairs from majority of the chromomeres and from each chromomere 1 to 9 loops may arise. The size of loops vary from an average of 9·5 μ in frog to 200 μ in newt. Ris (1960) has suggested that the loops are the integral part of chromonema which are extended in the form of major coils.

The function of such chromosomes includes the synthesis of *RNA* and protein by their loops (Miller, 1973) and probably the formation of certain amount of yolk material for the egg. Besides, the formation of several nucleoli due to the rings detached from the loops at specific loci has also been reported. The significance of such nucleoli is not known.

3. Supernumery Chromosomes

Known in the nuclei of certain plants and animals these are also called accessory chromosomes. They are relatively smaller in size than other two (described earlier) and are little unstable member of chromosome complement. They are different from other normal chromosomes as they do not show the activity of somatic non-disjunction and illumination and are randomly distributed.

4. Sex Chromosomes

Sex chromosomes are described under the chapter on Sex determination.

GENERAL QUESTIONS

1. Of all the structures in a cell, the nucleus is the most complicated. Describe critically, the ultrastructure and function of nucleus.

2. Summarise the present state of our knowledge regarding the structure of nucleus and chromosomes.

3. Write an essay on special types of chromosomes found in various types of cells.

4. Write short notes on, (i) Nucleolus, (ii) Balbiani ring, (iii) Chromosome morphology.

■ ■ ■

CHAPTER 5

THE CELL CYCLES AND CELL DIVISIONS

THE CELL CYCLES

The term 'cell cycle' is commonly used to describe the history of a cell from the time of its formation as a product of mitosis to the completion of its own division into two daughter cells. The different phases of the life cycle those occur in between have been recently referred by the letters G_1, S, G_2 and M. These abbreviations were originally used for the terms as such G_1 for gap 1, S for synthesis of *DNA*, G_2 for gap 2 and M for mitosis. While working on meristematic regions of bean root using radio autographic technique, Pele and Howard (1953) found that the period of synthesis of *DNA* is a well defined interval during the middle of interphase. They proposed that this time should be called the *S* period. Since they could not observe *DNA* synthesis in the cell either preceding or following *S* period, they referred to them as gaps one and two, respectively. Accordingly in between G_1 and G_2 phases, the various stages of mitosis occur. It would mean that after a cell has divided (M), there is a time of growth when no detectable *DNA* synthesis occurs (G_1 period) followed by *S* period and finally another time (G_2 period) when *DNA* synthesis is not traceable until the next cell division. Such remarkable divisions have been found in many types of cells.

This kind of cell cycle is almost of common occurrence in eukaryotic cells and is absent from prokaryotic cells. In bacteria, an example of prokaryotic cell, synthesis of *DNA* continues through most of the cell cycle. Why this kind of definite time of *DNA* synthesis is found in the organisms with a definite nuclear membrane is not clearly known. However, timing pattern is not universal. Baserga and Wiebel (1969) hold the opinion that the relative length of these periods vary some what in different species, in different tissues and even in the same cell under different culture conditions.

The period of G_1 and G_2 varies remarkably between different species. G_1 may be virtually absent in some cases. The S period in mammalian cells occur over 6 to 8 hours so is the case in higher plants. Kihlman (1966) states that total duration of cell cycle in *Vicia faba* and *Tradescantia paludosa* is 19·3 hrs and 17 hours, respectively (fig. 5.1).

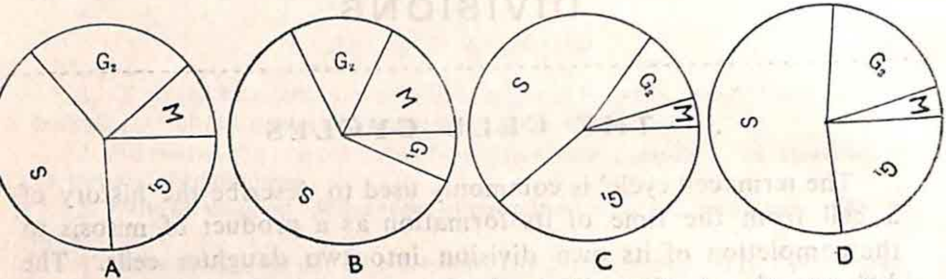


Fig. 5.1. Cell cycles. A—in *Vicia faba* (total time—19·3 hrs—G_1=4.9 hrs, G_2=4·9 hrs, M=2·0 hrs, S=7·5 hrs); B—in *Tradescantia paludosa* (Total time=17 hrs; G_1=1; G_2=2·5; S=10·5 & M=3·0); C—in Mouse fibroblast (total time=22 hrs) and D—in Chinese hamster fibroblast (total time=11·0 hrs).

The synthesis precess among other phases is complicated. Comings (1967) reported that not all the chromosomes replicate at a time. This invites a keen look on the problem. In human females, *x* chromosome does not begin replication until 2.5 hours after the other chromosomes initiate the S period and it continues for about 1.16 hours after most of others have finished. Even the replication of same *DNA* complement varies in different tissues of the same organism. In giant salivary cells of *Drosophila melanogaster*, S period lasts for about 8 to 14 hours while in the embryo cells, it consists of less than ten minutes. This may be due to variation in the number of replication sites, e.g. in mammalian cells, *DNA* molecules replicate simultaneously at several independent sites. The variation is also known about the rate at which single replication fork moves along a *DNA* double helix, e.g. in bacterial chromosome it's about 20 - 30 μ/min. while in He La cells 0·5 μ/min.

Such calculations and their interpretations have led to draw many interesting conclusions. For example, in human diploid cells, a single replication site moving at about 1μ/min. can synthesise only about 600 μ of helix during an S period of ten hours. Whereas when helix to be replicated during this period is about 174×10^4 μ, nearly

2900 replication sites distributed over 46 chromosomes would be needed. Each of these sites will be called replicon. These facts also show that *DNA* replication is controlled in a reproducible manner from one chromosome to the other in the same nucleus and even from place to place in the same chromosome.

However, the recent studies have opened several new facets of this problem. While working on *Vicia* Kihlman and Hartley (1967) observed that some *DNA* synthesis occurs in G_2 phase and in prophase of mitosis at a level that is undetectable by autoradiography. In such cases, the two periods G_2 and S may differ quantitatively rather than qualitatively. Similarly applying isotopic methods, Hotta, et. al. (1966) have also detected *DNA* synthesis both in G_2 phase and in prophase of meiosis in Lilly and *Trillium* anthers.

However, in the conventional cell cycle G_1 interval is commonly 8 to 10 hours the S phase, 6 to 8 hours, the G_2 interval 2 to 4 hours and mitosis about 1 hour. But several variations are known to this type. In the microspores of plant *Bellevalia*, *DNA* synthesis initiates immediately after mitotic phases which means that G_1 phase is undefinable.

Cell cycle and Cell surface

Mazia (1974) suggested that the switching of the different stages in cell cycle may be explained with the help of behaviour of cell membrane. It has been observed that membrane potential changes during cell cycle. The cell membrane is an active and responsive part of the cell and it decides what is inside and what is outside and what the outside does to the inside. He believes that the things outside a cell rather than passing through the membrane may induce some simple changes in a way that causes the membrane, in turn, to make changes in the cell interior.

The work on Chinese hamster has focussed some relevant light on this problem. Porter observed that during M phase cells are spherical and are strongly attached to the substrata they grow on. With the start of G_1 phase, cell begins to flatten and the cell surface exhibits bubble-like blisters and finger-like projections called microvilli. While with the progress of it (G_1 phase), cells become thin and active and present a ruffled appearance. The cell surface changes again during S phase and its complexion becomes smooth. Going through the G_2 phase, the cells become more thick, their surface again show microvilli and raffles and the blisters of G_1 phase are much less abundant.

CELL DIVISIONS

The basic object of any living being is to produce their own image and thereby to continue a species from generation to generation. We are simply tools of time and for a brief period in nature we come, play accordingly and after passing the treasure of nature, i.e., the reproducible capacity, we pass away.

The cell is the basic unit of life and division of the cell is the characteristic feature of every organism. It was first pointed out by Nageli (1846) that the new cells are always formed by the division of the pre-existing ones. Soon it was supported by Virchow (1855). Schleicher (1878) proposed that the main dividing structure of a cell is the nucleus. He called nuclear divisions as karyokinesis.

Besides many modifications in the fundamental pattern of cell divisions, the following three are important.

(i) Amitosis, (ii) Mitosis and (iii) Meiosis.

AMITOSIS

Also being called direct cell division or amitotic cell division and very simple division, it's common among unicellular animals like protozoans, certain fungi such as yeasts and in bacteria. During the process, the nucleus elongates and become dumb-bell shaped. A constriction in the middle of nucleus is produced which deepensl and ultimately it is broken into two daughter nuclei. Simultaneously, splitting of cytoplasm and cell membrane is followed. The splitted cytoplasm surrounds the each daughter nucleus.

MITOSIS

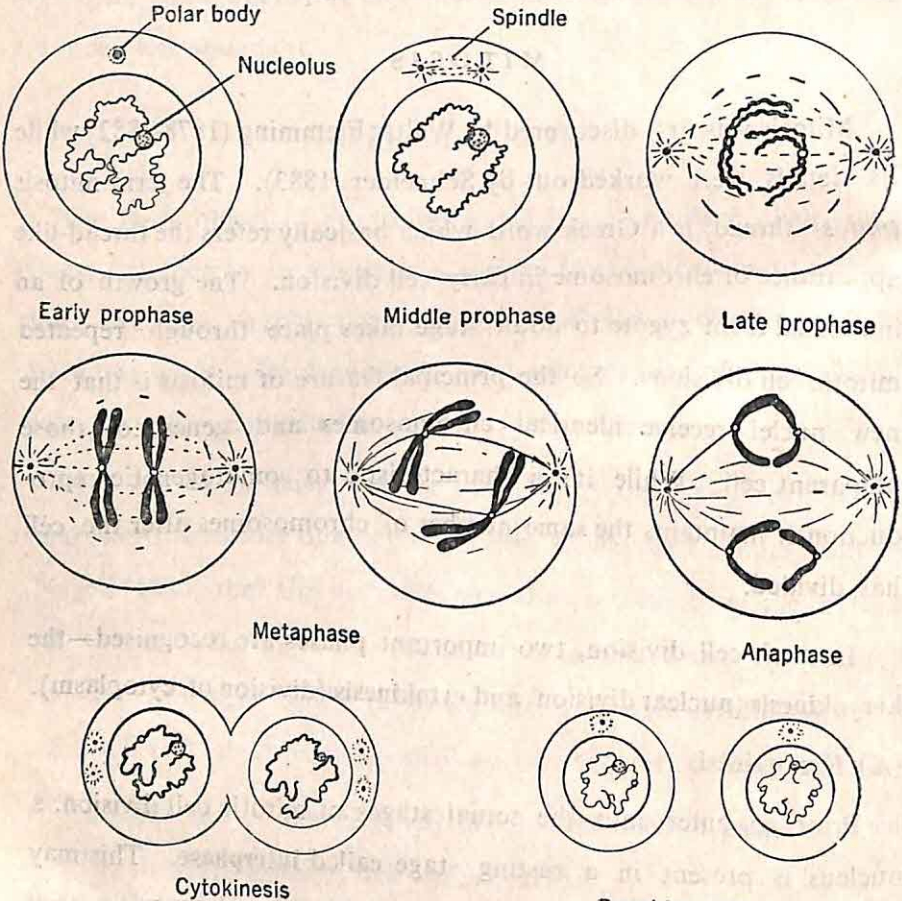
Mitosis was first discovered by Walter Flemming (1878-1882) while its details were worked out by Schneider (1883). The term mitosis (*mitos* = thread) is a Greek word which basically refers the thread-like appearance of chromosome in early cell division. The growth of an individual from zygote to adult stage takes place through repeated mitotic cell divisions. So the principal feature of mitosis is that the new nuclei receive identical chromosomes and genes to those of parent cell. While it's a characteristic to somatogenetic reproduction it maintains the same number of chromosomes after the cell has divided.

In each cell division, two important phases are recognised—the **karyokinesis** (nuclear division) and **cytokinesis** (division of cytoplasm).

(A) Karyokinesis

Prior to enter into the actual stages of mitotic cell division, a nucleus is present in a resting stage called **interphase.** This may however be referred as a critical period when the cell prepares itself for the division. The nucleus appears then as a dark staining body, the nucleolus being pronounced and the chromatids while showing minimum degree of condensation or coiling remain dispersed in the nuclear sap. In fact, interphase is the most active stage for the nucleus when it controls the synthesis of different types of enzymes to regulate metabolic processes and also it undergoes with the process of nucleic material *(DNA)* duplication. Three stages in the interphase nucleus have been recognised. They are G_1, S and G_2, each separately described under heading 'cell cycle'.

Besides interphase, four different stages—Prophase, Metaphase, Anaphase, and Telophase can be recognised in mitosis.



Early prophase Middle prophase Late prophase

Metaphase Anaphase

Cytokinesis Daughter cells

Fig. 5-2. Schematic representation of different stages of mitosis in animal cells.

1. PROPHASE—Being the longest stage of mitosis, it may last long from few minutes to several hours. In spleen of mouse it is completed in 21 minutes while in onion root in 71 minutes, in neuroblast cells of grasshopper in 102 minutes and in pea endosperm in about 40 minutes. At the beginning of prophase, the chromosomes appear as a thin, thread-like, uncoiled structure that becomes coiled, shortened and more distinct as the mitosis progresses through the prophase.

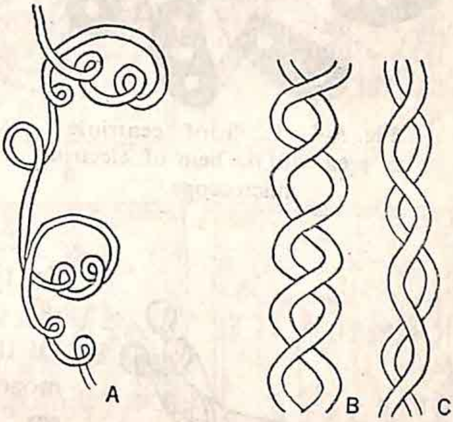


Fig. 5-3. Diagram showing prophase coiling. A—showing major or somatic and minor coilings, B—plectonemic coiling and C—paranemic coiling.

Another important feature of prophase is the longitudinal splitting of each chromosome into two sister chromatids. The two sister chromatids are attached only at the position of centromere. The coiling occurs in between these two sister chromatids which may be of two level—larger ones called somatic coils and smaller ones minor coils. In most cases, the pattern of coiling is plectonemic type in which chromatids are twisted about each other relationally so much that it is not easy to separate them (fig. 5-3). In a few cases, paranemic coiling may also be seen.

Towards the end of prophase spindle formation occurs. In case of animal cells, the centriole, a special structure close to nucleus, also divides into two daughter centrioles. Ultrastructure studies made on centriole reveal that a centriole is built up of nine obilquely oriented peripheral fibrils, each composed of three sub-fibrils. Structurally they are quite similar to basal body of flagellum. The division of centriole may be of two types. In one case, the divided centrioles separate and each shows an aster or astral rays and in between the two asters, a bundle of delicate spindle filaments appear. The centrioles along with aster continue to migrate towards their respective poles. While in the second case, the centrioles are already

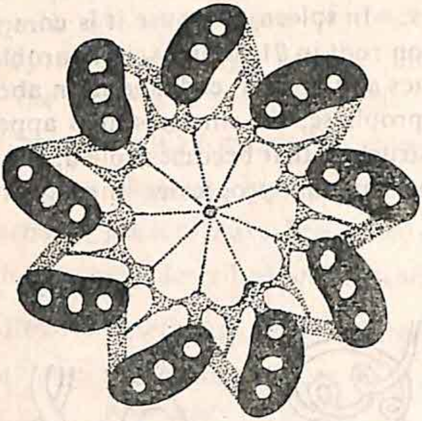


Fig. 5-4. T. S. of centriole as seen with the help of electron microscope.

polarised prior to nuclear division. Such mitoses are called amphiastral types being common to animals and a few lower plants (*Spirogyra* sp.) In contrast, plants show anastral type of mitoses.

Finally at the end of prophase, the nuclear membrane begins to disappear into the cytoplasm. Similarly nucleolus also disappears before the cell enters the metaphase.

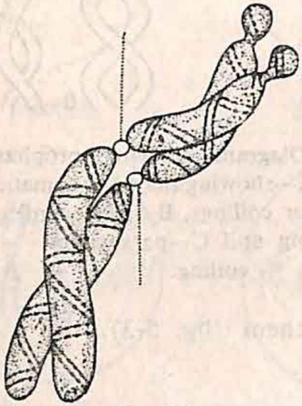


Fig. 5-5. Condition of chromosome as seen during metaphase.

2. METAPHASE—As described earlier that towards the end of prophase the spindle tubules begin to appear. Of these some are attached to chromosomes called chromosomic fibres and others are continuous from one pole to other called continuous fibres. The attachment of spindle tubules to the chromosomes is instrumental in directing the chromosomes to the cell equator. Simultaneously the centromeres of the chromosomes divide so that each sister chromatid has its own centromere.

Thus, in metaphase, the duplicated chromosomes come to lie in the equatorial plate of cell.

3. ANAPHASE—Anaphase begins with the movement of chromosomes towards their respective poles. At this stage as soon as the centromere becomes functionally double (divided), sister chromatids separate from each other due to a kind of repulsive force produced between the two centromeres. Each separated chromatid with its own centromere is now called chromosome. The movement of chromosomes

towards opposite poles of spindle is the characteristic feature of anaphase and is believed to be supported by contraction of spindle fibres.

In anaphase, . the chromosomes assume typical shapes depending upon the position of their centromeres. They may look like V shaped,

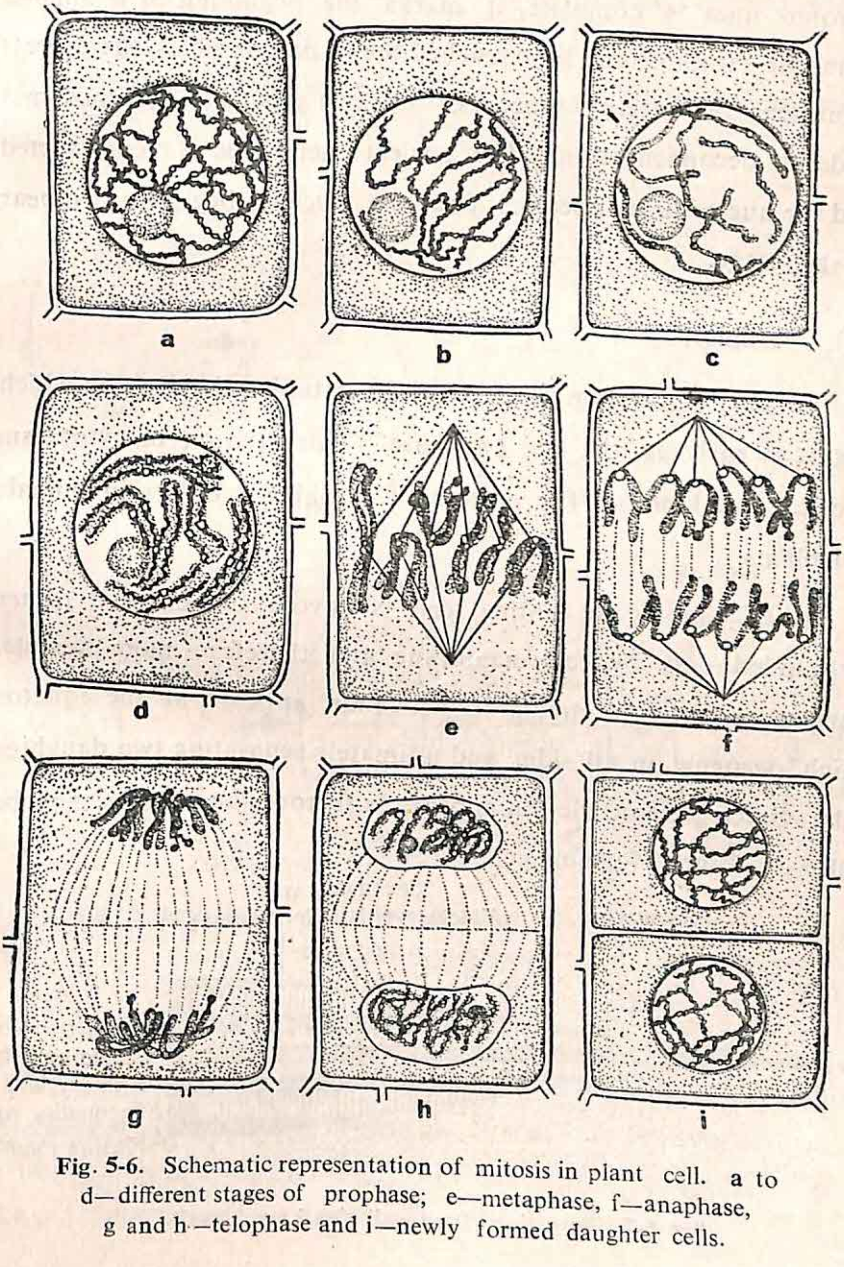


Fig. 5-6. Schematic representation of mitosis in plant cell. a to d—different stages of prophase; e—metaphase, f—anaphase, g and h—telophase and i—newly formed daughter cells.

J-shaped or I-shaped in case of metacentric, sub-metacentric and acrocentric chromosomes, respectively.

4. TELOPHASE—When the polar movement of two groups of chromosomes is complete, it marks the beginning of telophase. After the chromosomes have reached the respective poles, the process of nuclear reconstruction occurs. The two groups of chromosomes undergo decondensation. The nuclear membrane is reconstructed and the nucleolus reappears. Thus, the nucleus begins to reappear at this stage.

(B) Cytokinesis

The nuclear division is followed by cytoplasmic division which begins as early as the late anaphase. This division of cytoplasm is called cytokinesis. The pattern of cytokinesis differs in animals and plants.

(i) *In animals* : An animal cell is devoid of cell wall rather surrounded only by cell membrane and therefore more flexible. During cytokinesis a circular constriction appears at the equator which deepens on all sides and ultimately separating two daughter cells. Since the constriction appears as furrow, the process may be called cytokinesis forming cell furrow.

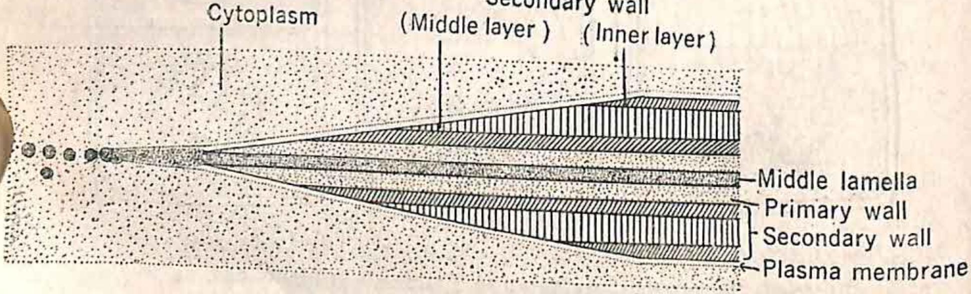


Fig. 5-7. Formation of cell wall after karyokinesis.

(ii) *In plants* : In plants, the cell wall is rigid and during cytokinesis a cell plate is laid down in the centre which is completed towards the periphery. The formation of cell plate occurs with the deposition of certain cytoplasmic droplets or microspheres which is soon followed by the deposition of materials of primary and secondary walls. The process is also called cytokinesis by cell plate formation.

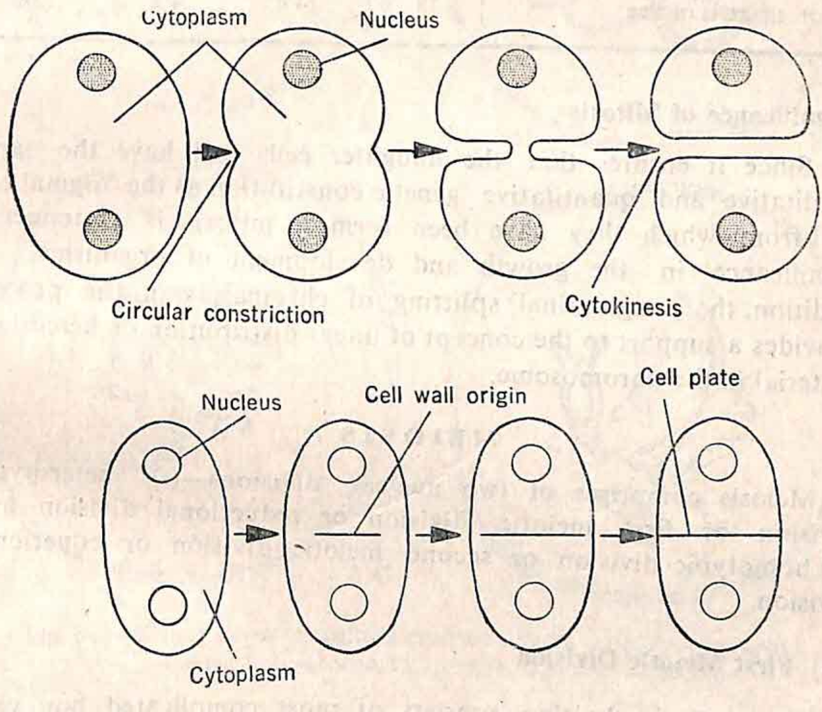


Fig. 5·8. Pattern of plate formation in animal cells (upper row) and in plant cells (lower row).

Time Sequence in Mitosis

The time taken by one mitotic process differs in pretty many ways depending upon the organisms, tissues, temperature and other environmental and chemical factors in question. The process may take place within 10 minutes to several hours. However, the perusal of following table verifies the statement.

Examples	*Time in minutes (Duration)*			
	Prophase	*Metaphase*	*Anaphase*	*Telophase*
Cells of Spleen of Mouse	21	13	5	4
Neuroblast cells of Grasshopper	102	13	9	57
Root tip cells of Onion	71	6·5	2·4	3·8
Root tip cells of Pea	78	14·4	4·2	13·2

Significance of Mitosis

Since it ensures that the daughter cells will have the same qualitative and quantitative genetic constitution as the original cell had from which they have been formed, mitosis is of immense significance in the growth and development of organisms. In addition, the longitudinal splitting of chromatids in the process provides a support to the concept of linear distribution of hereditary material in the chromosome.

MEIOSIS

Meiosis comprises of two nuclear divisions—(i) heterotypic division or first meiotic division or reductional division and (ii) homotypic division or second meiotic division or equational division.

(A) First Meiotic Division

First meiotic division consists of most complicated but very important stages of cell division since the process of reduction in chromosome number occurs within its various steps. Basically it is divided into 4 stages—Prophase I, Metaphase I, Anaphase I and Telophase I but its prophase I needs special mention.

1. PROPHASE I—Longest among all other stages prophase I differs from mitotic counterpart in several respects which can be seen in next few steps. Owing to its complication and also for the sake of convenience, the stage is further divided into five sub-stages as follows.

(i) *Leptotene* : Leptotene is not much distinct from the mitotic prophase. In this stage, the nucleus enlarges in size and the chromosomes begin to appear as a long, uncoiled, thread-like, loosely interwoven structure. The arrangement of chromosomes is often irregular. Occasionally among animals, particularly in certain insects, a tendency of polarisation is also seen in which case the chromosomes have their ends drawn together at a point on nuclear membrane near the centriole. This peculiar arrangement is sometimes referred as **bonquet**. Likewise in certain plants, particularly in *Lilium*, a phenomenon called **syninzesis** is seen where the chromosomes are clumped to one side of nucleus and there being no chromatin material seen in the rest part of the nucleus. It is believed that

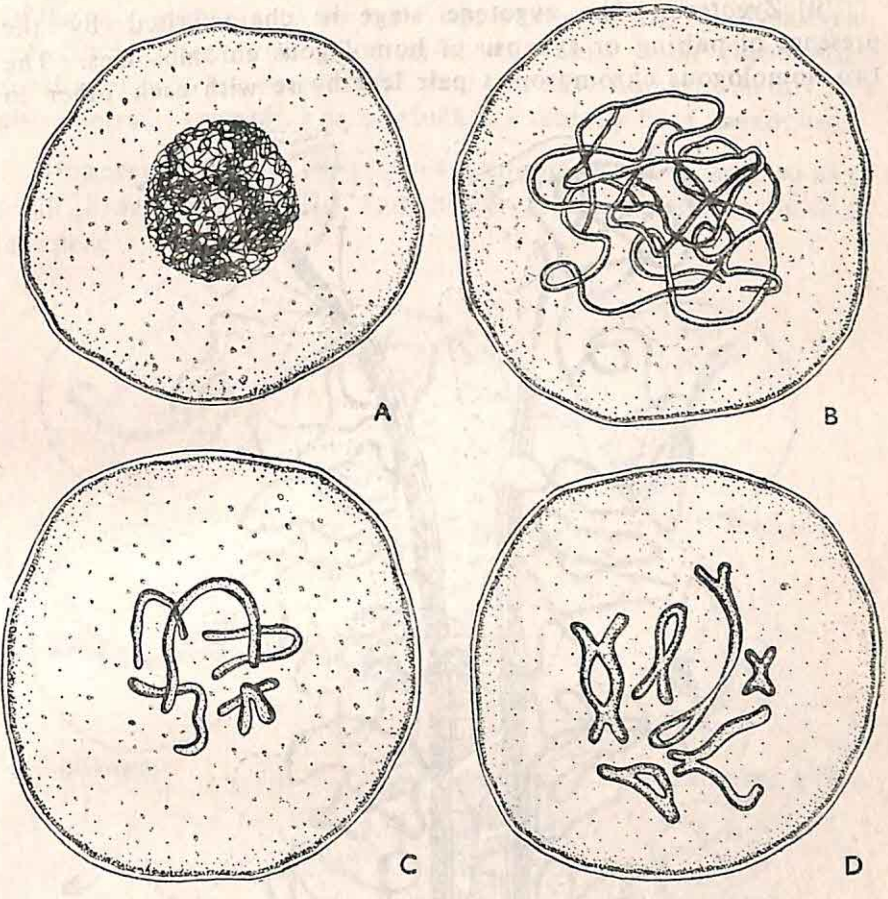


Fig. 5-9. Various stages of meiosis (redrawn from microphotographs). A—leptotene, B—zygotene, C—pachytene and D—diplotene.

duplication of chromosomes occurs in this stage which could not be seen under microscopes. The nucleolus remains conspicuous.

(ii) *Zygotene* : The zygotene stage is characterised by the presence of pairing or synapsis of homologous chromosomes. The two homologous chromosomes pair lengthwise with each other in

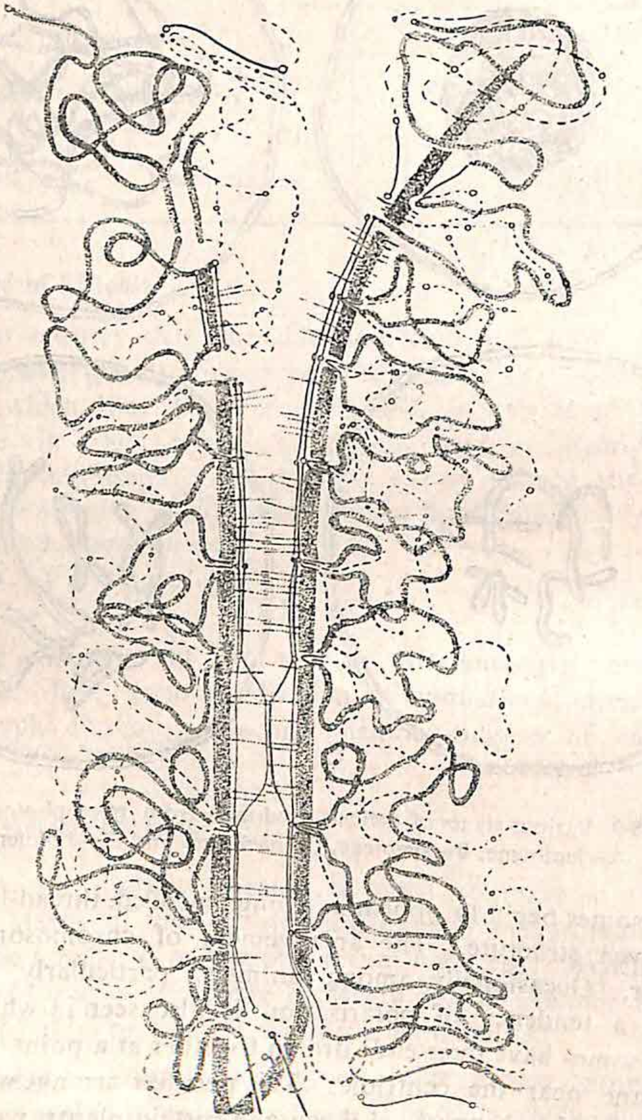


Fig. 5-10. *Diagram to show the probable assembly of a synaptinemal* complex from two axial cores. The central element may represent *DNA* spun out on either sides of two homologues. (Redrawn from Moens, 1968). (Stage—starting zygotene to pachytene)

a zipper-like fashion starting from centromere or at the chromosome ends. The pattern of pairing is characteristic to species in question. Of the two synepticiss chromosomes, one is paternal and other maternal, collectively these are called **bivalents**. Since each chromosome consists of two chromatids, a bivalent will have four chromatids. It's also referred as **tetrads**, a term which has recently been abandoned.

However, coiling in chromosomes continues in this stage so as to result chromosomes shorter and thicker in comparison to those of leptotene.

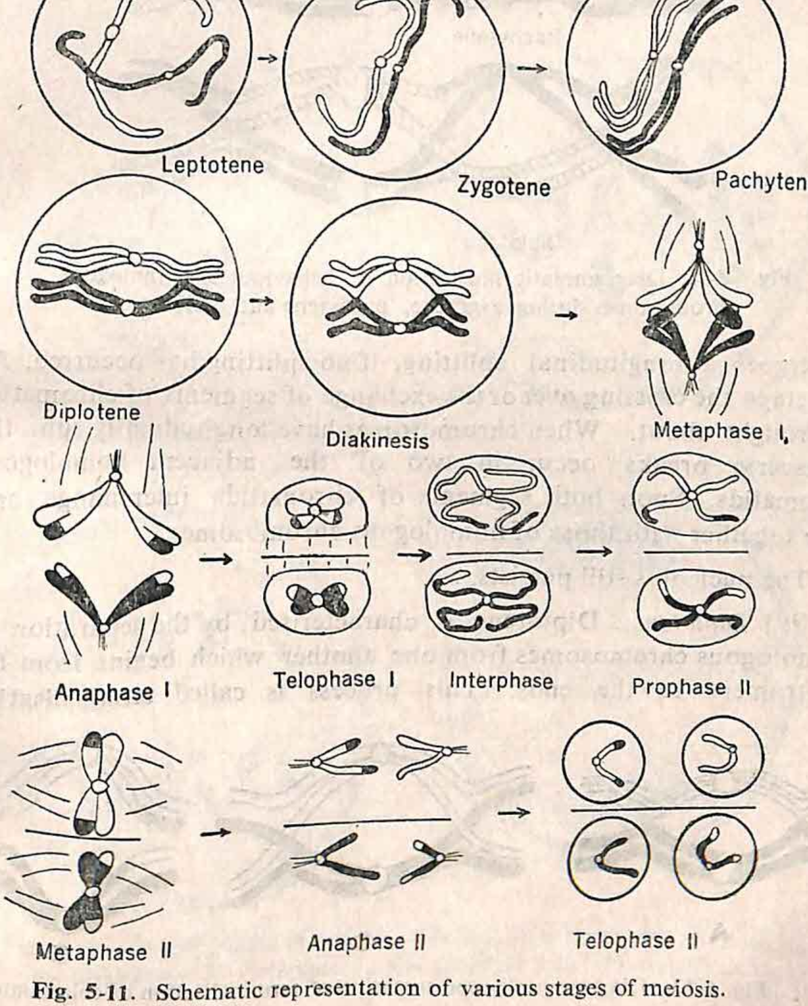


Fig. 5-11. Schematic representation of various stages of meiosis.

(iii) *Pachytene* : Pachytene begins with the end of chromosomal synapsis. The chromosomes are further shortened and coiled. At the height of pachytene, the paired chromosome may twist about one another as relation coils and further the two chromatids of each chromosome may also coil around each other. Each chromosome undergoes a longitudinal splitting, if no splitting has occurred. At this stage the crossing over or the exchange of segments of chromatids is brought about. When chromosomes have longitudinally split, the transverse breaks occur in two of the adjacent homologous chromatids. Soon both segments of chromatids interchange and fuse together with those of homologous chromosomes.

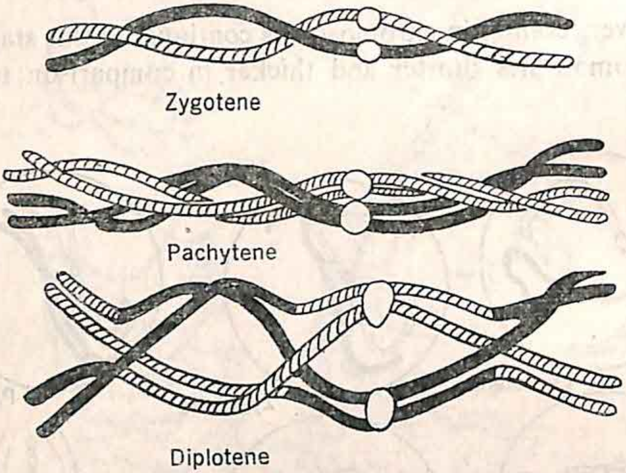


Fig. 5-12. Diagrammatic illustration of behaviour of homologous chromosomes during zygotene, pachytene and diplotene.

The nucleolus still persists.

(iv) *Diplotene* : Diplotene is characterised by the separation of homologous chromosomes from one another which begins from the centromere to the ends. This process is called **terminalisation**

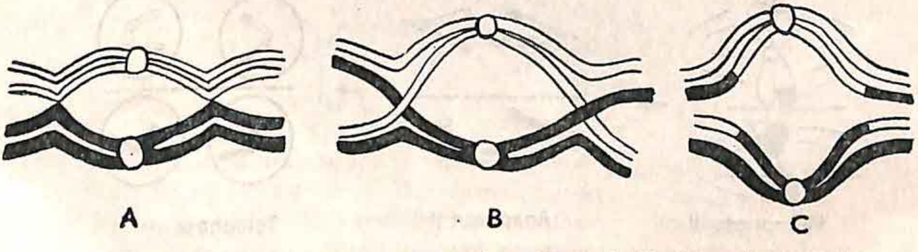


Fig. 5-13. Diagrammatic representation of terminalisation of chiasmata.

(Darlington, 1930). The name diplotene is characteristic to this stage as the dual nature of bivalent becomes distinct due to such separation. The separation is not complete as the two homologous chromosomes remain held together at certain points along the length. Such points of contact between the two homologous chromosomes are called **chiasmata** which represent the place of actual crossing over. With the process of terminalisation, these chiasmata move towards the end of the chromosomes. (Crossing over mechanism is discussed in a separate chapter.)

The nucleolus and nuclear membrane at this stage begin to disorganise. Nucleolus may still persist but nuclear membrane can not be seen. The process of contraction of chromosome still continues.

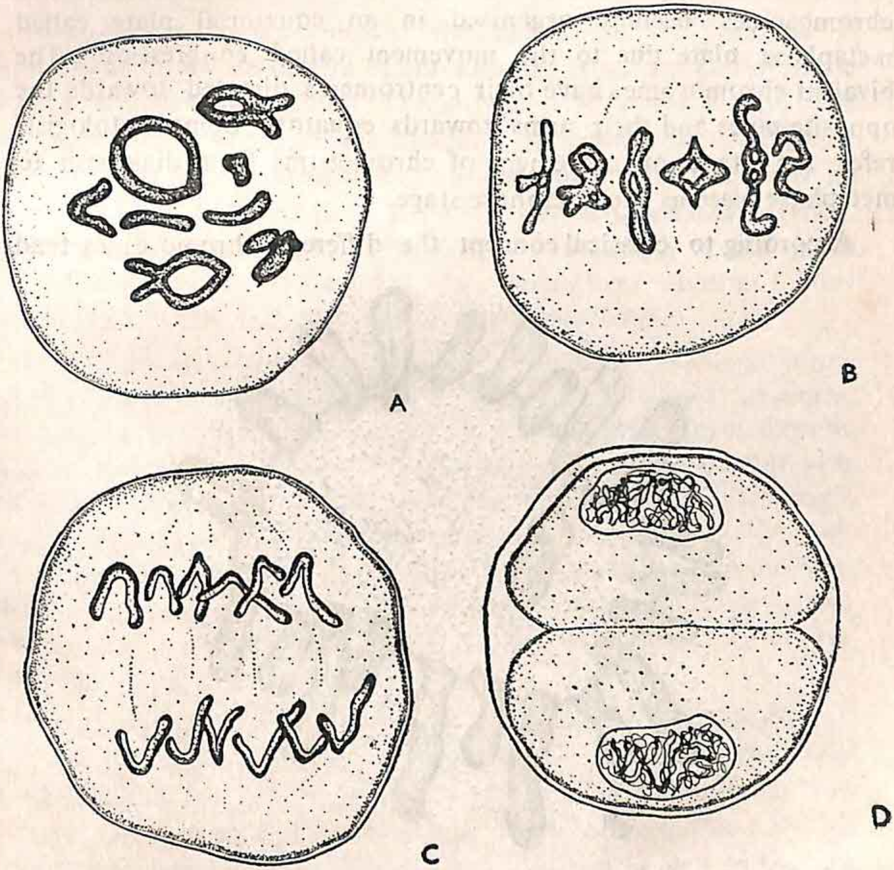


Fig. 5-14. Various stages of meiosis (redrawn from microphotographs). A—diakinesis, B—metaphase I, C—anaphase I and D—telophase I.

(v) *Diakinesis* : The diakinesis can be distinguished from diplotene by the presence of more contracted state of bivalents. Due to further contraction and terminalisation, the bivalents may appear as rounded bodies evenly scattered through out the cell or have the tendency to be at the periphery near the nuclear envelope. The nucleolus also disorganises and is no longer visible at the end of this stage.

Occasionally in case of a longer chromosome, the terminalisation process is not complete and some of the chiasmata may appear intercalary.

2. METAPHASE I—The chromosomes are now most condensed with smooth outline. The spindle fibres appear and bivalents become attached to them at the centromere of chromosome. The chromosomes become organised in an equatorial plate called metaphase plate due to the movement called congression. The bivalent chromosomes have their centromeres directed towards the opposite poles and their arms towards equator. Some cytologists refer the stage of movement of chromosome from diakinesis to metaphase plate as prometaphase stage.

According to classical concept the different chromosomes tend

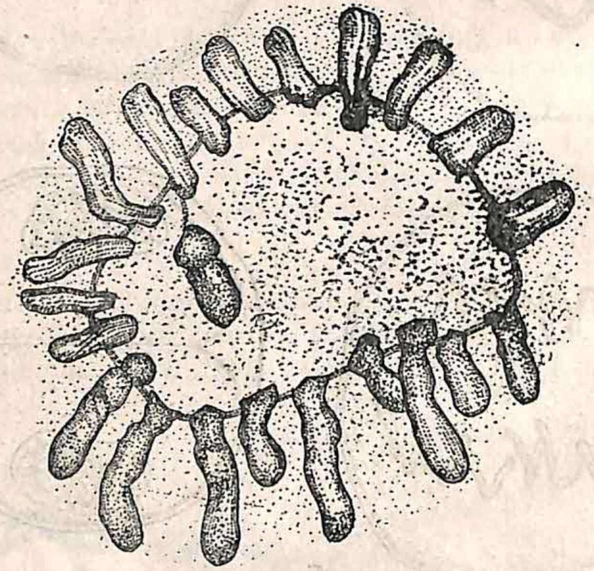


Fig. 5-15. Oogonial cell of *Didelphis*. Chromosomes at metaphase plate showing radial configuration and interconnectives. (Redrawn from Tsu, 1967)

to occupy characteristic positions on their metaphase plate. Among many insects, the larger chromosomes lie near the rim of the circular plate while smaller ones lie in the middle. This specific organisation is ascribed to unknown 'attractive forces' operating between different chromosomes. However, some earlier workers such as Sutton (1903), Gross (1904), Wilson (1909, '25) observed the presence of chromosome-to-chromosome connectives in certain cases. This fact has been further supported by electron microscopic studies. Tsu (1967) while working on opposum *Didelphis* and Kangaroo rat tissue culture cells, observed the ring of chromosomes interconnected by a fine strand linking the centromeres. The chromosome-to-chromosome connectives are strikingly evident in *Didelphis* where visible connectives can be seen passing from centromere to centromere of the 22 chromosomes (fig. 5-15)

3. ANAPHASE I—Like mitosis, the anaphase I of meiosis is chracterised by the movement of chromosome from equatorial plate to the poles but chromosomes of meiosis are different in nature. The sister chromatids do not separate because centromere does not divide and these go to the same pole (unlike mitosis). Since there is no separation of sister chromatids, it's called **non-disjunctional** division. As a result, half of the total chromosomes of a nucleus would go to one pole and rest half to the other, i.e., reductional division. Now each group contains haploid number of chromosomes.

In the process, the two chromatids of the chromosome widen apart except at the centromere and assume a V-shaped structure. The shapes however vary and depend on position of centromeres. Each chromosome besides its double number of chromatids also differs in composition. While the two chromatids maintain their initial nature, the other two are mixed (due to crossing over). The two centromeres, one of paternal and other of maternal chromosome are directed towards a different pole. As soon as chromosomes move towards pole, the chiasmata, if so far remained, loosen and finally free the separating chromosomes.

4. TELOPHASE I—Telophase I is characterised when the anaphase chromosomes conglomerate at poles. In the actual process, the chromosomes elongate by loosening of the coils. The nucleolus and nuclear membrane reappear. However, cytokinesis may or may not occur at this stage and there may be two cells or a cell with two nuclei and a common cytoplasm. If the cell wall is formed it's called successive division and if not it's simultaneous division.

Interkinesis : Two such formed haploid daughter nuclei undergo a short resting stage which occurs in between telophase I and prophase II. In certain cases, such as in *Trillium* sp. the chromosomes while retaining coiling at telophase pass on to prophase II. However, the interkinesis, also called interphase, is either very short or may be entirely absent.

(B) Second Meiotic Division

It's a simple mitotic division and therefore may be called meiotic mitosis. The characteristic features of different stages of mitosis have been discussed earlier. The stages may be called—prophase II, metaphase II, anaphase II and telophase II.

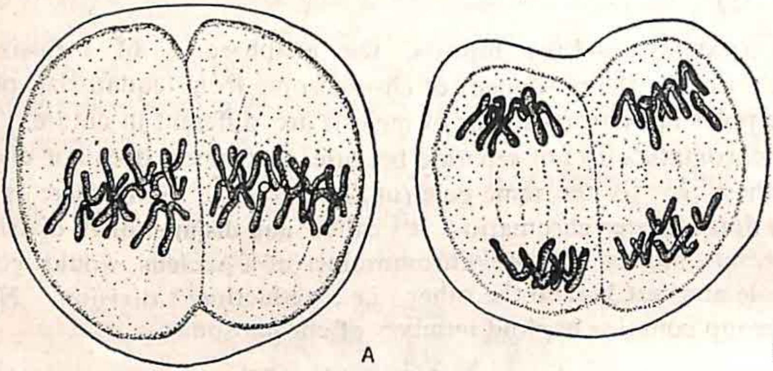


Fig. 5.16. Stages of meiosis. A—metaphase II and B—anaphase II. (Redrawn from microphotographs)

The chromosomes of prophase II are already double, i.e., each one has two sister chromatids with a single functional centromere. Shortly these chromosomes arrange on metaphase plate where their centromeres split. Each separated part is called chromosome (daughter chromosome). With the help of spindle fibres, these chromosomes soon begin to move towards the two poles (anaphase II). Since the chromosomes were doubled each group will contain similar number of chromosomes. The division therefore may be called an equational or a homotypic division. Simultaneously, telophase II set in which is soon followed by cytokinesis. Cytokinesis whether successive or simultaneous type will give rise to four haploid cells.

While visualising the different stages of meiosis, it is found that four nuclei or cells develop from a single germinal cell. Each has a haploid number of chromosomes. Of the four, two contain basically paternal and other two maternal chromosomes.

COMPARISON BETWEEN MITOSIS AND MEIOSIS

Mitosis	**Meiosis**
1. Resting stage preceding mitosis is considerably long.	1. Resting stage is shorter.
2. Prophase is short stage and without substages.	2. Prophase is long and composed of leptotene, zygotene, pachytene, diplotene and diakinesis.
3. During the preceding resting stage, the chromosome longitudinally split into two sister chromatids. Therefore, from the early stage, chromosomes appear as double threads.	3. During preceding prophase, chromosomes do not appear as double thread rather as a single thread (leptotene).
4. The homologous chromosomes are not attracted to each other.	4. The homologous chromosomes are attracted to each other and form bivalents. The chromosomes then split into sister chromatids and at four strand stage crossing over takes place. During crossing over, there is exchange of segments of chromatids between the maternal and paternal chromosomes. Chromosomes later on separate and chiasmata appear. Depending upon the number and size of chiasmata, the chromosomes give characteristic appearance.
5. During metaphase, the centromere of each chromosome divides and the sister chromatids move towards the opposite pole. Thus the number of chromosomes remains maintained.	5. Without the division of centromere, homologous chromosomes move towards opposite poles. Therfore, the number of chromosomes at any place is reduced to half, i.e., reduction in chromosome number occurs at this stage. It is called metaphase I.

6. The chromosomes which begin to move towards opposite

6. Chromosomes at metaphase I are so oriented that either

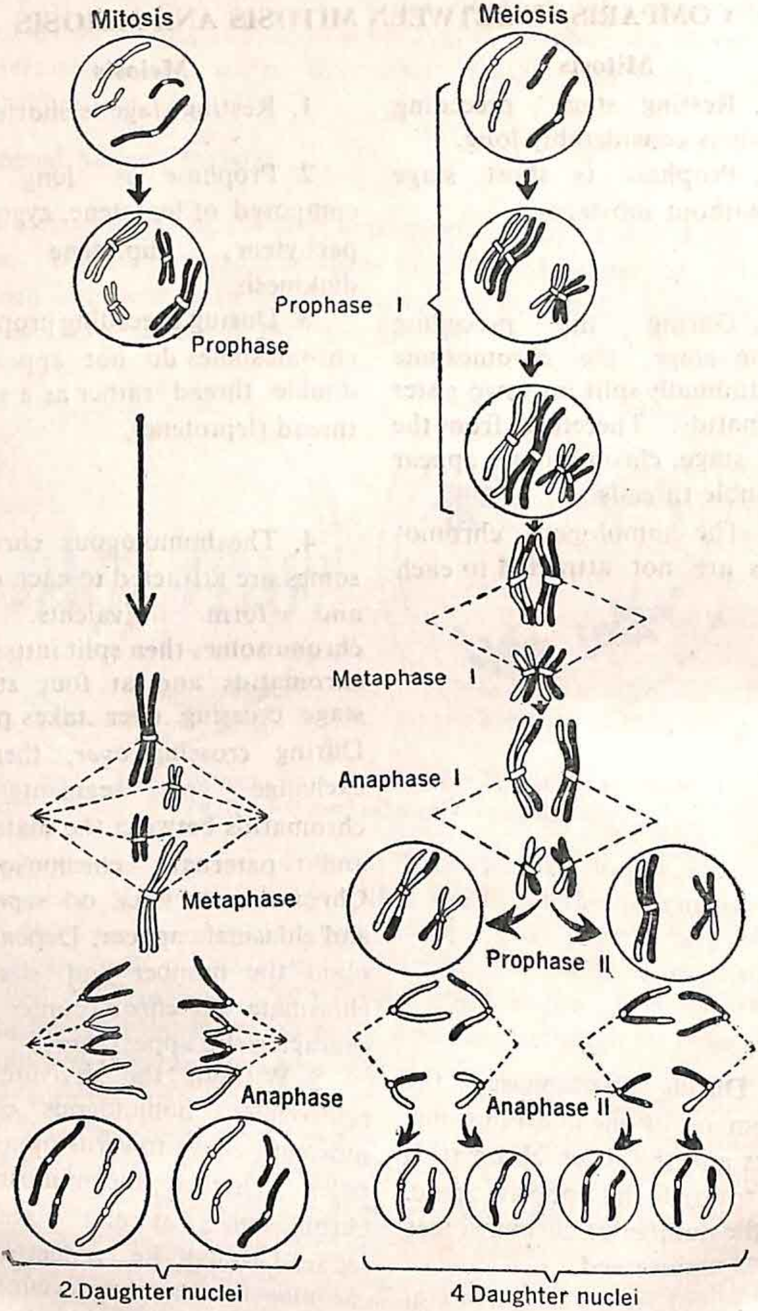


Fig. 5.17. Schematic comparison between mitosis and meiosis.

poles are of both maternal and paternal types (due to longitudinal division). Therefore chromosomes of both the poles would be identical.	paternal or maternal chromosome only goes to one pole. Therefore, chromosomes of both the poles could not be identical.
7. The movement of chromosomes towards the poles is due to repulsion between the parts of divided centromere. The two centromeres belong to same chromosome.	7. The repulsion occurs between the centromeres of homologous chromosomes. The two centromeres are of different chromosomes.
8. Anaphase mechanism is similar.	8. Anaphase I mechanism is similar to that of mitosis.
9. The two daughter nuclei formed at telophase have same number of chromosomes as that of parental cell.	9. The chromosome number is reduced to half.
10. As a result, by the end of division, neither chromosome number nor quality of chromosomes has changed.	10. The number and quality of chromosomes are changed.
11. No further division occurs and therefore only two daughter cells are formed.	11. This division is followed by mitotic division (similar to mitosis) and therefore 4 daughter cells, each with reduced number of chromosomes, are formed.

PHYSIOLOGY OF CELL DIVISIONS

Out of many tempting facets of cytogenetics, cell division is of chronic interest as something wonderful within a cell can be visualised with the help of most easily available compound microscopes. The universal occurrence of such stages in eucaryotes adds further interest and invites to keen cyto-geneticists and inquisitive students to maintain some prudent concept of this magic fact. Undoubtedly, the available concerned literature offers conclusive explanation to simple thinkers, yet if seen the various steps with deep concentrations, each one creates a new problem. Some of them being possible to discuss within the scope of the book have been taken up here. Of course, these would serve as prenote only.

The fact that cell division is a classical name of chromosome reproduction initiates a question in mind to explore the temporal controls in chromosome reproduction. To ask "what is the molecular basis of chromosome reproduction ?" might appear a precise and clean question. Actually it is more undefined and confusing. Had chromosome reproduction been a single event, it must have been the most ideal question. The fact is that it is not. Hubert Stern (1972) while replying to this question said that "the degree to which a molecular mechanism elucidates a particular cellular event can be no better than the degree to which that event has been defined." He suggested that to the various facets of chromosome reproduction molecular analyses could be applied to more restricted targets.

What regulates the cell division is probably present in both cytoplasm as well as in the nucleus. A cytoplasm from the dividing cell is capable to induce chromosome replication when in contact with an otherwise non-replicating nucleus. It's not the sole factor but is subject to other determinants that supply a temporal ordering of regional replications both within and between chromosomes. Specific sites that control the initiation of chromosome replication are also known present within the chromosomes and it is visualised that a form of message must exist between different regions of a chromosome and between different chromosomes in order to account for the fine aspects of the pre-determined temporal pattern of chromosome reproduction.

Problems when visualised at the cellular level offer several specific opportunities for molecular explanations. At the very first hand the capacity of cytoplasm to induce chromosome replication only at the time of nuclear division provides tentative conclusion that the onset of *S* phase is due to some cytoplasmic transformations. The fact was looked upon the involvement of provision of essential enzymes for *DNA* replication. However, studies made on the enzyme changes in association with *DNA* replication do not support this interpretation and the problem remains a total mystery at the molecular level.

Meiosis is considered a pattern for achieving a reduction in chromosome number. Undoubtedly it is a common method but by no means the sole pattern specially in insects where in many species unusual is usual. However, to the cytologists more important is to learn the centromere behaviour in meiosis. Two points need clean explanations—(i) the timing of effective centromere division and (ii) its physical association with spindle fibres. An explanation to first point based on the fact that the centromere division is a result of *DNA* replication in the centromere region and that the timing of this replication is affected by mechanisms similar to those in other regions of chromosome has not been substantiated by any kind of chemical evidence. Moreover, cytological studies indicate that the centromere is a compound body prior to metaphase. The second point provides even less scope for speculation. Stern (1970) holds the opinion that chromosome fibre running to the pole of the spindle formed at the centromere is reasonably well established and that this characteristic property is retained by the centromere even in the fragmented chromosomes. Since the chromosome is considered to be a linearly differentiated *DNA* templete, it is difficult to think of the chromosome as to have some special site for the formation of such special structures. If not, then whether centromeres have a specific mechanism for synthesising some special protein which might serve either as monomers for fibre formation or the centromeres are the site of polymerisation of pre-existing monomers into a fibril ? These questions are extremely difficult to answer.

Geneticists have other problems of major interests such as phenomena of pairing and crossing over. At the same time it should come in the mind that the occurrence of pairing and crossing over is not unique to meiosis. Somatic pairing occurs regularly in certain insects and is also known in the salivary gland chromosomes of *Drosophila* and crossing over, in fact, between the sister chromatids is common in mitosis where it is called sister strand exchange. Crossing over occurs most frequently in viruses than higher organisms. However, crossing over occurs in meiosis when homologous chromosomes are brought into close proximity. Should it be considered as a mere coincidence of pairing or the synchronised occurrence of chromosomes ? When this phenomenon is compared with mitosis an explanation comes out itself. The prophase in mitosis extends for about an hour while that in meiosis it extends for days or even weeks. The extended time interval must be associated with a sequence of distinctive changes in the chromosomes. Obviously, it's a presumption just as crossing over is presumed to occur during pachytene because pairing is completed at the end of zygotene and persists no later than the beginning of diplotene. This argument is quite logical but without any direct evidence.

Similarly the problems like—What initiates meiosis ? What causes synapsis ? What causes diplotene opening ? Does coiling have any role in meiosis ? What is the exact mechanism of crossing over ? How are the chromosomes brought and organised in a metaphase plate ? etc. are various delicate questions with insufficient replies.

Let us look some of the problems explained on the basis of circumstantial evidences produced during establishing temporal correlation between macromolecular syntheses and cytological events. Chromosomes in general consist of nucleic acid (*DNA*) and proteins.

DNA : If synthesis of *DNA* is inhibited at the beginning of zygotene, the cells show no further development. These cells fail to form synaptinemal complex. It means that synthesis of *DNA* is essential to the chromosome pairing. However, if *DNA* synthesis is inhibited at different intervals of zygotene, in each case further progress stopped. Thus *DNA* synthesis is not only essential to the initiation of pairing but also to the continuation of the pairing. When considered the absolute amount of *DNA* synthesised during meiosis, it has been found that 99·6 per cent is synthesised at *S* phase while rest at the different stages of cell division. Stern (1970) states that amount formed during zygotene and pachytene are very small even though they have major functional significance to meiosis. Besides, the formation of synaptinemal complex, *DNA* synthesis during zygotene is essential to the structural integrity of each of the chromosomes.

Protein : In fact the total cell division is the result of various drama of proteins. If protein synthesis is inhibited at any stage of meiosis, the effects are always adverse and the result is either partial or complete meiotic arrests. Whether a few or all the proteins synthesised during cell division are essential to maintain the various acts of meiosis has also been unravelled and found that among many proteins produced only one or two are essential and that these alone are responsible for meiotic arrest. It has been found that when protein synthesis was inhibited for 2 or 3 days at the early stage of zygotene, the inhibitor acted as though it stopped all the processes and when it is inhibited in late zygotene or early pachytene for same period, the chromosomes proceed through pachytene but when they reach diplotene no chiasmata were formed. These findings permitted to visualise that proteins responsible for chromosomal pairing are synthesised at different time whereas proteins essential to chiasmata formation at some other time. The absence of chiasmata could be explained by the failure of chromosome pairs to hold together long enough to reach the crossing over stage.

The study based on *DNA* and protein syntheses reveals that *DNA* synthesis is essential to the occurrence of pairing and protein synthesis to its stabilisation. Furthermore, stabilisation appears to be necessary condition for crossing over and that crossing over between homologs is not an accidental consequence of replication in two juxtaposed *DNA* strands but a deliberately regulated event following the completion of pairing.

GENERAL QUESTIONS

1. What is cell cycle? What are G_1 and G_2 phases? Discuss briefly the various types of cell cycles met in plants.
2. Write an essay on physiology of cell division.
3. "Mitosis constitutes an example of fundamental cell division." Comment upon the statement.
4. "Meiosis is lengthy and complicated but most important type of cell division." Discuss critically and also mention its significance.
5. Give a comparative account of mitosis and meiosis.
6. Write short notes on (i) Pachytene chromosomes, (ii) Chiasmata, (iii) Bivalents, (iv) Syneptinemal complex, (v) Chromosome-to-chromosome connectives.

■ ■ ■

CHAPTER 6

LINKAGE AND CROSSING OVER

LINKAGE

Exceptions to Mendel's law of independent assortment was first discovered by Bateson and Prunnet (1906) when they found that two pairs of alleles in *Lathyrus odoratus* did not assort independently. They explained it on the basis of **coupling** (genes tend to enter in the same gamete) **and repulsion** (genes tend to enter in the different gametes) **phenomenon.** Soon the phenomenon of linkage was discovered in *Drosophila* by Morgan (1910) and was studied in details. He and his coworkers established four groups of linkage corresponding to the four pairs of chromosomes containing several hundred genes. The genes here behaved as if they are to be inherited in a unit or in a block. This phenomenon in which the characters are inherited in the form of groups is referred as **linkage** and genes locating in such chromosomes are called **linked genes**. Morgan noted that coupling and repulsion and linkage are the two aspects of a single phenomenon called linkage.

Linkage in Drosophila

Morgan discovered two recessive mutants—**black** (black body) and **vestigeal** (wings reduced to useless stubs) in *Drosophila.* When a cross between two was made, in F_1, normal fly (grey bodied with long wings) was produced and in F_2, the typical ratio of 3 : 1 was obtained. When F_1 is back crossed with double recessive black vestigeal (test cross), the resulted ratio is quite different from expected 1 : 1 : 1 : 1 as it should have been in other normal cases. The results of such cross are shown in Fig. 6.1 which elucidates the fact that two pairs of genes *Bb* and *Vv* do not assort independently. As a result, the parental combinations (83%) exceed greatly than recombinations (17%).

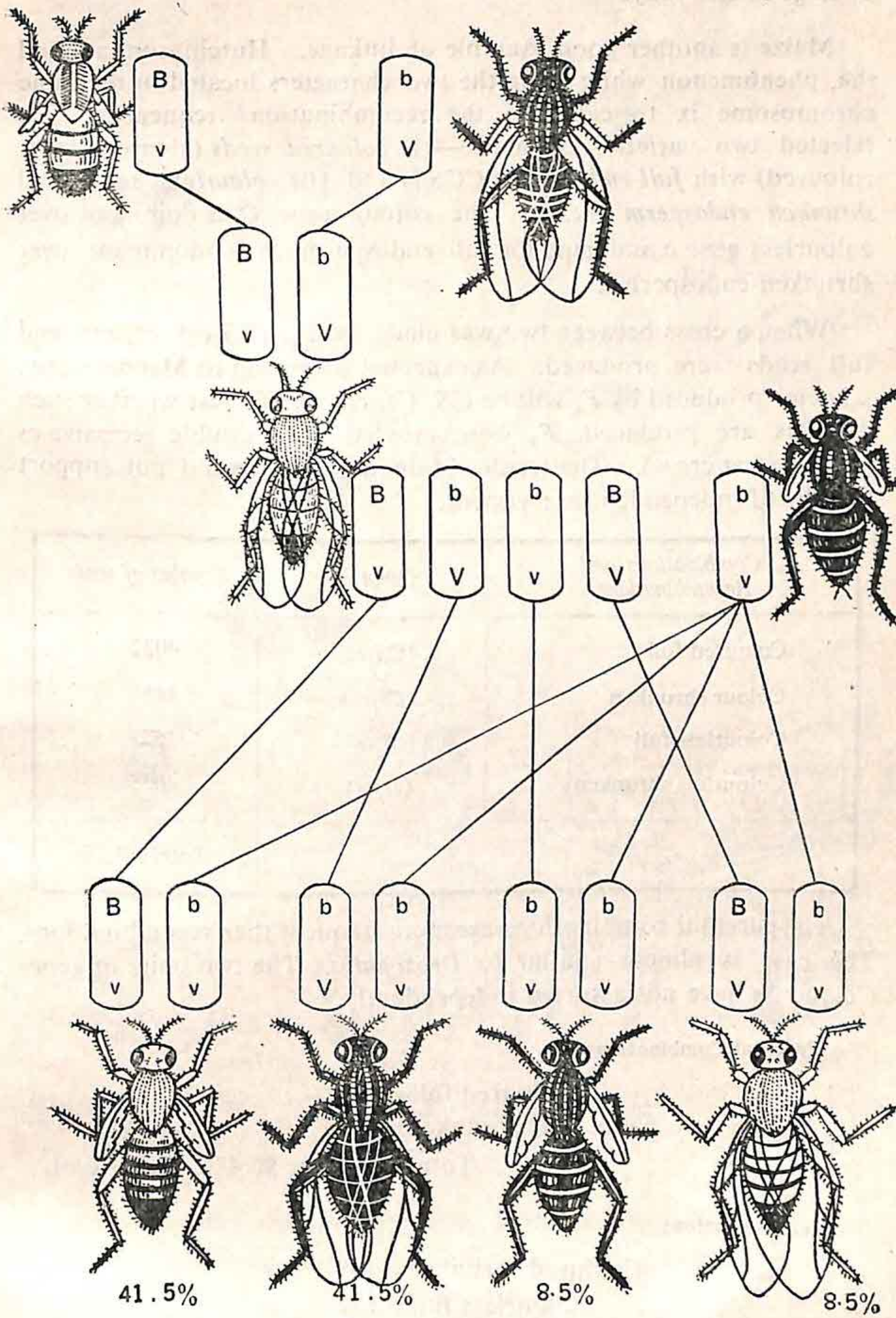


Fig. 6.1. Pictorial representation elucidating linkage in *Drosophila melanogaster*.

Linkage in Zea mays

Maize is another good example of linkage. Hutchinson studied the phenomenon while using the two characters located in the same chromosome ix to calculate the recombination frequencies. He selected two varieties of maize—(i) *coloured seeds* (aleurone layer coloured) with *full endosperm* (*CCSS*) and (ii) *colourless seeds* and *shrunken endosperm* (*ccss*). The colour gene *C* is dominant over colourless gene *c* and gene for full endosperm *S* is dominant over shrunken endosperms.

When a cross between two was made, in F_1, (*CS*/*cs*) colour and full seeds were produced. As expected according to Mendel's law, gametes produced by F_1 will be *CS*, *Cs*, *cS*, *cs*. To test whether such gametes are produced, F_1 were crossed with double recessive *cs* gamete (test cross). The result obtained as follows did not support the law of independent assortment.

Combinations and Recombinations	*Genotype*	*Number of seeds*
Coloured full	(*CS*/*cs*)	4032
Colour shrunken	(*Cs*/*cs*)	149
Colourless full	(*cS*/*cs*)	152
Colourless shrunken	(*cs*/*cs*)	4035
		Total=8,368

The parental combinations are more frequent than recombinations. The case is almost similar to *Drosophila*. The two pairs of genes *Cc* and *Ss* have not assorted independently.

Parental Combinations

Coloured full = 4032
Colourless shrunken = 4035
Total = 8067 or 96·4% of the total.

Recombinations

Coloured shrunken = 149
Colourless full = 152
Total = 301 or 3·6% of the total.

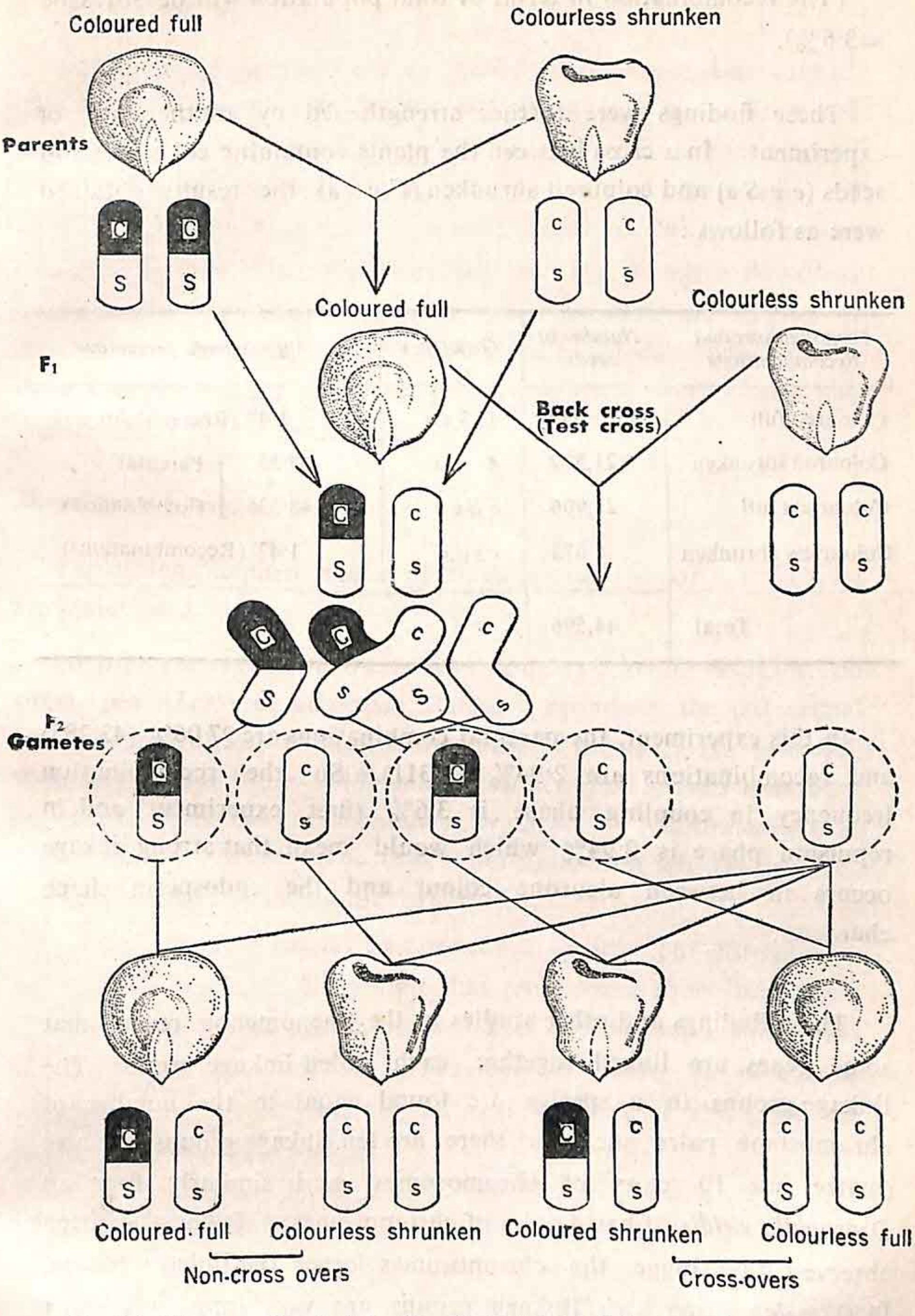


Fig. 6.2. Diagram illustrating the chromosomal linkage and crossing over in *Zea mays*.

(The recombination in terms of total population will be 301/8368 =3·6%).

These findings were further strengthened by another set of experiment. In a cross between the plants containing colourless full seeds (*c c S s*) and coloured shrunken (*C c s s*), the results obtained were as follows :

Combinations and Recombinations	*Number of seeds*	*Genotypes*	*Approximate percentage*
Coloured full	639	*C S c s*	1·47 (Recombinations)
Coloured shrunken	21,379	*C s c s*	48·53 Parental
Colourless full	21,906	*c S c s*	48·53 Combinations
Colourless shrunken	672	*c s c s*	1·47 (Recombinations)
Total	44,596		

In this experiment, the parental combinations are 97·06% (43,285) and recombinations are 2·94% (1,311). So the recombination frequency in coupling phase is 3·6% (first experiment) and in repulsion phase is 2·94% which would mean that strong linkage occurs in between aleurone colour and the endosperm shape character.

These findings and other studies in the phenomenon reveal that some genes are linked together, each called linkage group. The linkage groups in a species are found equal to the number of chromosome pairs such as there are ten linkage groups in maize (maize has 10 pairs of chromosomes) and similarly four in *Drosophila viridis* (it has 4 pairs of chromosomes). It has also been observed that bigger the chromosomes larger the linkage groups, In *D. melanogaster*, two linkage groups are very long, one short and fourth one is intermediate between the two.

Classification of Linkage

Linkage may be complete or incomplete. In complete linkage, the linked characters appear in two or more generations. They are rare in nature and are known in *Drosophila* and other insects. Incomplete linkages occur due to accidental breakage of chromosome as in maize. Some special kinds of linkages are the sex linkage and interchromosomal linkage. Sex linkage is described under chapter sex-linked inheritance. Interchromosomal linkage is referred to the type where the members of a pair of linked genes show a further linkage to other genes, thereby resulting into the linkage series in the chromosome.

Theories on Linkage

Explaining the phenomenon of linkage, two important theories are maintained.

(i) DIFFERENTIAL MULTIPLICATION THEORY : While working on sweet pea (*Lathyrus odoratus*) Bateson proposed the differential multiplication theory to explain the cause of linkage. He proposed that it was due to the "*differential multiplication of cells containing different combinations of characters or genes*". Multiplication in such cases is faster. The faster multiplication will result in the modification of Mendelian dihybrid ratio.

(ii) CHROMOSOME THEORY OF LINKAGE : It was put forward by Castle and Morgan. They state that genes which show linkage are situated on the same chromosome. The chromosomal substances however bind these linked genes together during the process of inheritance. It would obviously mean that the closer the gene the stronger will be the linkage.

Importance of Linkage

Many cases of linkage are known among the plants and animals. Besides those observed in *Drosophila* and *Zea mays*, a few examples of linked genes are given in the following table :

Name of the plant	*Linked characters*
Sorghum	(i) White seedling and red coleoptile. (ii) Yellow seedling and waxy endosperm.
Zea mays	(i) Sugary endosperm and tunicate ear. (ii) Sugary endosperm and white seedling. (iii) Yellow endosperm and yellowish leaf. (iv) Yellow endosperm and white seedling. (v) Yellow endosperm and male sterility. (vi) Pericarp colour and fine stripped leaf.
Gossypium	(i) Corolla colour and lint index. (ii) Lint colour and lint length. (iii) Pollen grain and nectaries. (iv) Hairy lint and light brown lint. (v) Curly leaf and khaki lint colour.
Oryza sativa	(i) Colour and weight of grain. (ii) Sterility and growing period. (iii) Yield and anthocyanin pigment. (iv) Stigma colour and leaf sheath colour.

The extensive studies of linkage have led to develop most of the modern genetical concepts and it has helped variously in the improvement of plants through hybridisation and selection programmes. It has also facilitated the congregation of several desirable characters in a plant through plant breeding.

Besides, linkage has helped in maintaining several parental characters together. In nature, it checks the appearance of new recombination. It is often referred that *linkage serves as a factor of racial and specific cohesion rather than as a barrier between species and between races.*

Occasionally linkage acts as a marker gene and is most frequently used as prenote of experimentations. It is visualised that the presence of qualitative characters such as leaf shape, corolla colour, grain colour etc., if linked with quantitative characters such as big panicles, lint index, yield, weight of grain etc., will speak about the incurrence of quantitative characters quite earlier. A very common example is witnessed in rice where the inheritance of anthocyanin pigment acts as a marker for yield and colour of grain as a marker of weight of grains.

Linkage in many cases presents great difficulties and sometimes it makes impossible to bring the desired economically important characters in an organism. For example, *Gossypium arboreum* is high ginnert with poor lint quality but other species is low ginnert with fine lint qualities. The scientist wanted to bring the high ginnert with fine lint quality together but linkage has wasted their arduous attempts. Even today such combination could not be achieved. Similarly in South Indian rice a combination of short duration and high yield could not be achieved.

CROSSING OVER

The term crossing over was coined by Morgan and Cattell (1912) to represent the events leading to genetic recombination between linked markers. It may also be defined as "the mechanism of recombination of the genes due to interchange of chromosomal segments at the time of pairing". According to this phenomenon there is an exchange of segments of homologous chromosomes including linked genes at meiosis. As a result, new recombinations are produced. Crossing over is well known in maize, pea, mice, man and others.

Mechanism of crossing over

Cytological studies made reveal the fact that crossing over phenomenon takes place at the four strand or tetrad stage of chromosome during prophase of meiosis. At this stage, each of the paired homologous chromosomes splits longitudinally into two chromatids which remain attached together by the undivided centromere of the chromosome. Chiasmata are formed between the two chromatids, one each from a homologous pair of chromosomes and the other two chromatids preserve the original characters. Which of the two chromatids are involved in chiasmata formation is difficult to determine earlier. During non-disjunction of the chromosome, these chiasmata break and the broken chromatids fuse

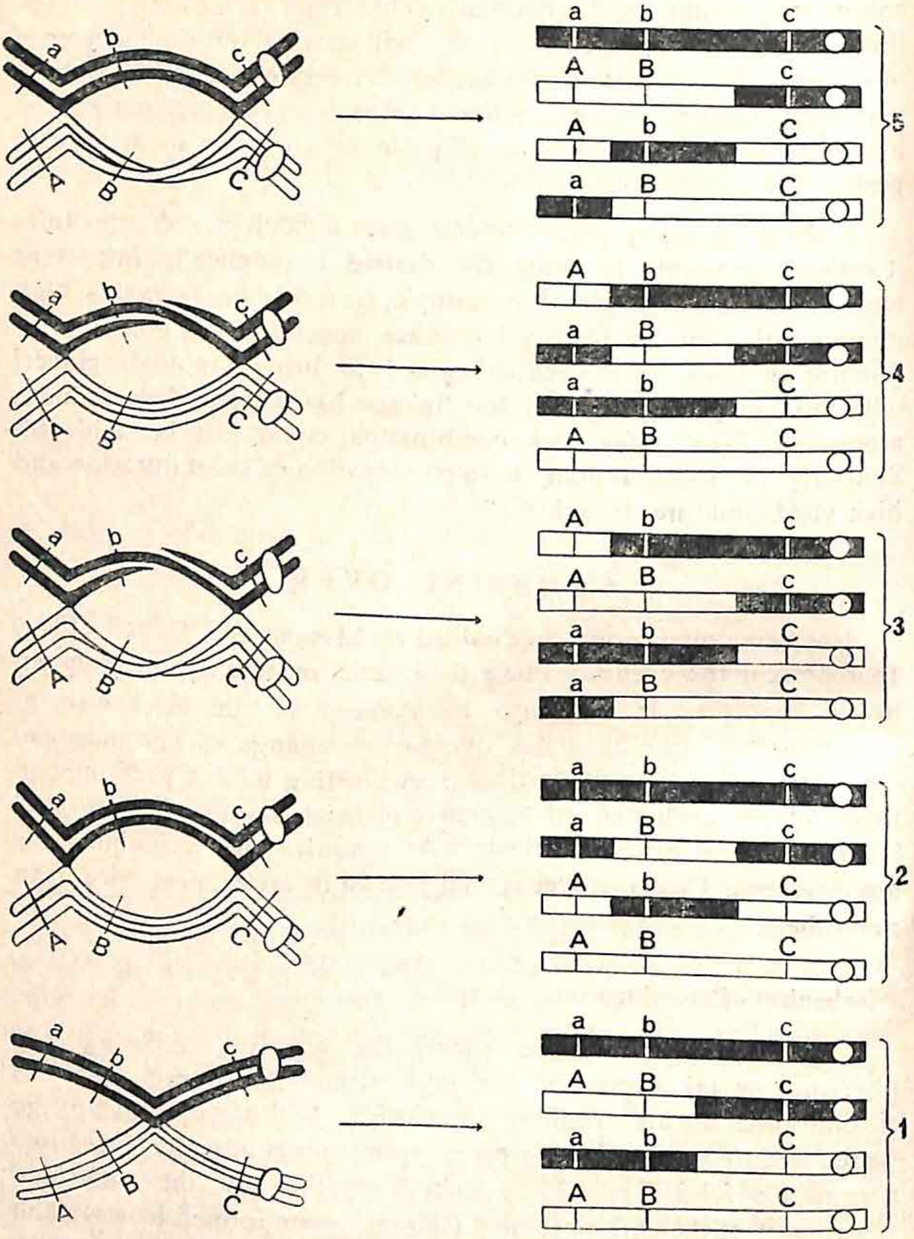


Fig. 6.3 : Diagram showing fate of chromosome bivalents with single and double chiasmata—1. Single chiasma ; 2. Two strand double cross over; 3. Four strands double cross over; 4. Double chiasmata—three strands involved and 5. Three strands double cross over.

together in a new fashion. They are so fused as to maintain the exchange of parts of chromatids between the two chromosomes of a homologous pair. Such exchange of chromosome segments during meiosis is called **crossing over.**

The actual mechanism of crossing over is not so simple as explained. Many theories have been proposed to elucidate the concept of crossing over and chiasmata formation. A few are described here.

1. THE CLASSICAL HYPOTHESIS—Proposed by Sachs (1932), this theory assumes that division of each chromosome into two chromatids occurs in early diplotene stage or late pachytene stage. The origin of chiasmata is then possible and there is a change of a pairing partner. The chiasma does not represent a cross over, rather crossing over results when a chiasma is ruptured and the broken end of chromosomes brings about recombination.

It is so organised that at one region the two chromatids of a chromosome remain associated while on the other side of a chiasma each pair of chromatid would consist of one paternal and one maternal threads.

Classical theory has been supported by most cytologists because it did not require breakage and rejoining of the chromosomes.

2. JANSSENS' HYPOTHESIS—While working on sperm formation in the amphibian *Batrachoseps attenuatus*, Janssens (1909) proposed that prior to the formation chiasmata, the homologous chromosomes remain loosely coiled round with each other and at intervals they make contact so as to penetrate the other until they break and they rejoin in new ways paternal to maternal or *vice versa*. The other two chromatids of a chromosome remain intact so as to give rise to the typical chiasmata with two chromatids crossing each other and two not. It may occur in two stages as explained in Fig. 6.4.

He further argued that the purpose of meiosis is to produce four haploid nuclei all of different genotypes. Later he emphasised that division should be considered as **chiasma type** rather than containing heterotypic and homotypic divisions in meiosis. According to this concept, there would be segregation of some factor differences at each division of meiosis in contrast to the view that segregation occurs at heterotypic or first division of meiosis.

He also pointed out that this theory provides an escape from the dilemma of the existence of a larger number of freely assorting allelomorphic characters than there are pairing of chromosomes.

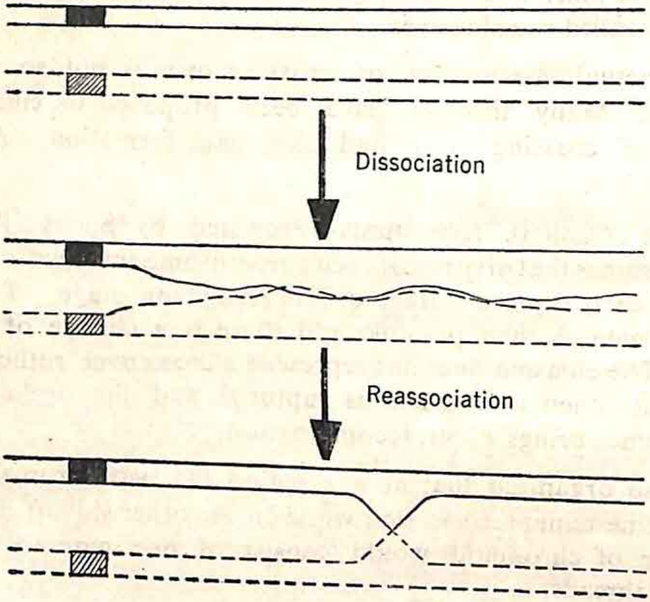


Fig. 6.4 : Illustration of the hypothesis. The two chromatids of a chromosome dissociate from one another. After this reassociation takes place which involves one of the chromatids of homologous chromosome.

3. TORSION THEORY—Darlington (1937) proposed "Electrostatic theory of chromosome behaviour" and postulated the existence of electrostatic forces of attraction and repulsion. During the phase of attraction two homologous chromosomes are attracted to each other as a pair to satisfy a two by two association. The paired equilibrium and longitudinal cohesion become disturbed at the time of formation of chromatids. Shortly, the attraction is replaced by repulsion which forces them apart but since they are relationally coiled about each other, a torsion is developed which eventually breaks two non-sister chromatids. Torsion is relieved by the movement of broken chromatids and when the broken ends rejoin. If the rejoining is in between two non-sister strands, crossing over will result.

The presence of such forces have not been demonstrated experimentally.

4. BELLINGS' HYPOTHESIS—Bellings (1933) proposed that crossing

over occurs between the new chromatids during the period of their formation. New chromomeres are formed first alongside their respective sister chromomeres and the interconnecting fibres are formed later. If relational coiling exists, the inter connecting fibres may sometimes be formed between non-sister chromatids. Thus cross over is produced.

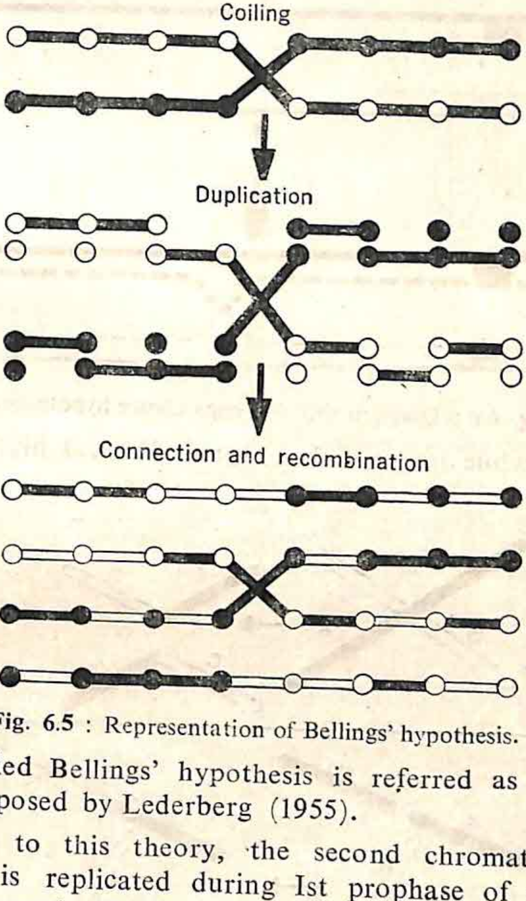


Fig. 6.5 : Representation of Bellings' hypothesis.

The modified Bellings' hypothesis is referred as copy choice theory as proposed by Lederberg (1955).

According to this theory, the second chromatid of each chromosome is replicated during Ist prophase of meiosis (just prior to pachytene). During the synthesis of *DNA*, the new helix may copy a non-sister helix rather than a sister helix to produce a recombination in a very short segment.

The recent researches have proved a factual mistake in it that *DNA* synthesis occurs even before the onset of meiosis, i.e., at least quite before the synapsis.

Some other theories worth to mention are the contact first theory and breakage theory of crossing over. In the former, the chromatids

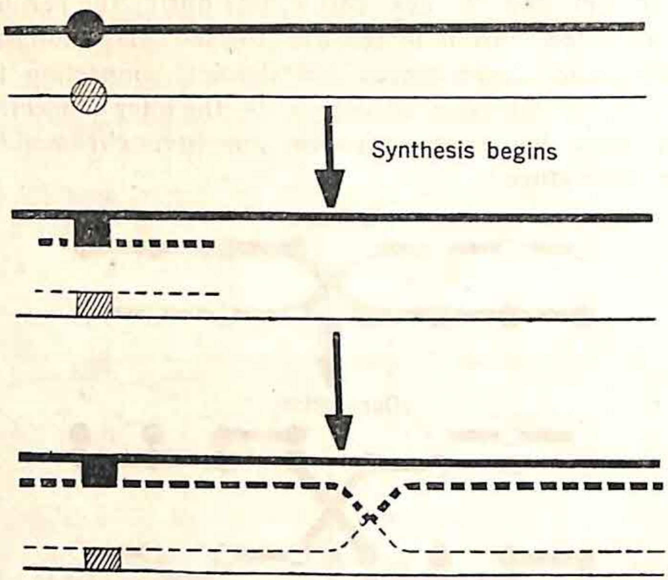


Fig. 6.6 : Diagram showing copy choice hypothesis.

contact first while in later, the chromatids break first, as explained in figure.

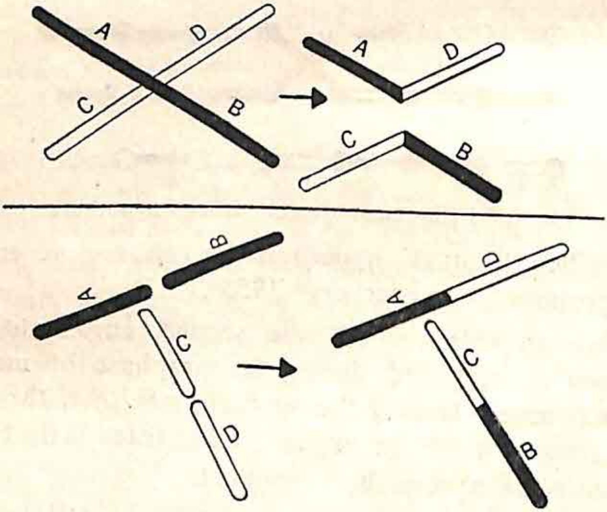


Fig. 6.7 : The upper part explains contact first theory of crossing over while lower part the breakage theory of crossing over.

Chromosomal maps

Chromosomal map means a linear graphic representation of a chromosome in which genes belonging to a particular linkage group are plotted according to their relative distances. Sturtevant (1911) was the first biologist who constructed the map. Later, the well established chromosomal map of *Drosophila* was prepared by Bridges.

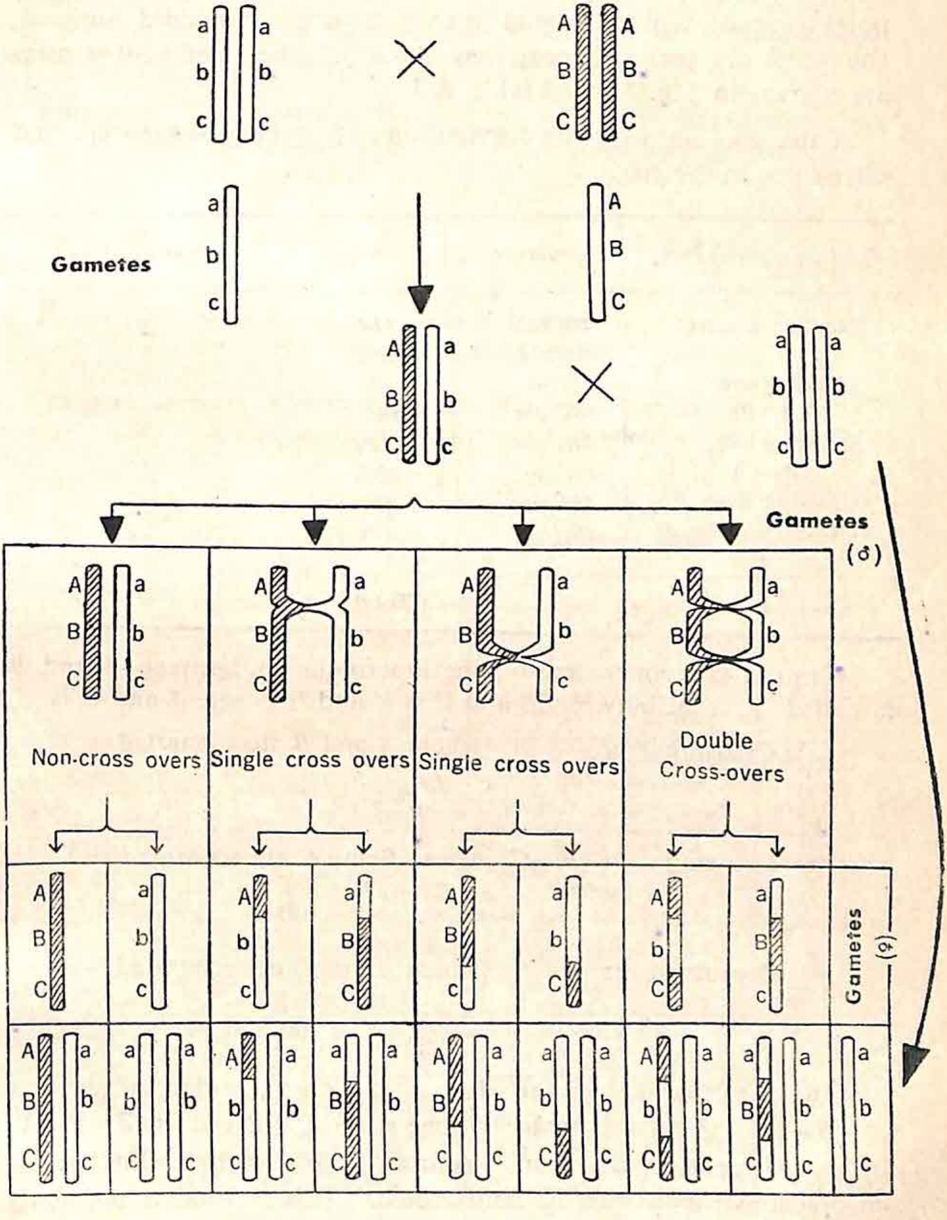


Fig. 6.8 : Three-point test cross which involves *A*, *B* and *C* hypothetical genes.

The construction of chromosomal map is possible by studying the relative frequency of linkages and recombinations. For example, three genes A, B and C are linked together. They may be present in either of the sequences such as $A-B-C$, $A-C-B$ or $B-A-C$, all A, B and C may be present in the middle or at ends. The main point in study will be to find out which gene is in middle. Suppose, the genes are present linearly as $A-B-C$, the results of crosses are shown in Fig. 6.8 and table. 6. I.

Table 6.I—Showing the distribution of frequency of genotype and phenotype in the cross.

Position of cross over	*Genotype*	*Phenotype*	*Supposed frequency*
Non-cross overs	*ABC/abc*	*ABC*	*a*
	abc/abc	*abc*	*b*
Crossing over (*A*—*B*)	*Abc/abc*	*Abc*	*c*
	aBC/abc	*aBC*	*d*
Crossing over (*B*—*C*)	*ABc/abc*	*ABc*	*e*
	abC/abc	*abC*	*f*
Crossing over (*A*—*D*) and (*B*—*C*)	*AbC/abc*	*AbC*	*g*
	aBc/abc	*aBc*	*h*
		Total	*T*

If in the experiment recombination frequency between A and B is called X, that between B and C is Y and between A and C is Z,

(i) Crossing over (A—B) (where A and B are separated) $=X$

$$=\frac{c+d+g+h}{T}\times 100$$

(ii) Crossing over (B—C) (where B and C are separated) $=Y$

$$=\frac{e+f+g+h}{T}\times 100$$

(iii) Crossing over (A—C) (where A and C are separated) $=Z$

$$=\frac{c+d+e+f}{T}\times 100$$

The result will indicate that if $Z=X+Y$, order of gene is $A-B-C$; if $Z=X-Y$, order of gene is $A-C-B$ and if $Z=Y-X$, order of gene is $B-A-C$. Adopting this method linkage or chromosomal map can be constructed. This method is popularly called **three-point cross method.**

Based on three point method Hutchinson (1922) constructed the linkage map of chromosome of *Zea mays*. He selected three characters, namely, coloured aleurone (*C*) versus colourless aleurone (*c*), full endosperm (*Sh*) versus shrunken endosperm (*sh*) and non-waxy endosperm (*Wx*) versus waxy endosperm (*wx*). The results of the cross are shown as follows :

Assuming that linear order is *C*, *sh* and *Wx*, three recombination values *C – sh*, *sh – Wx* and *C – Wx* will be worked out.

Parents C sh Wx/C sh Wx (coloured, shrunken, nonwaxy) X c Sh wx/c Sh wx (colourless, full, waxy)

Gametes C sh Wx c Sh wx

F₁ C sh Wx/c Sh wx (coloured, full, nonwaxy)

Text cross C sh Wx/c Sh wx (coloured, full, nonwaxy) X c sh wx/c sh wx (colourless, shrunken, waxy)

	Gametes (female)	**Gametes (male)** c sh wx	
No crossing over	C sh Wx	C sh Wx/c sh wx (coloured, shrunken, nonwaxy)	5,246
	c Sh wx	c Sh wx/c sh wx (colourless, full, waxy)	
Single crossing over (C-sh)	C Sh wx	C Sh wx/c sh wx (coloured, full, waxy)	229
	c sh Wx	c sh Wx/c sh wx (colourless, shrunken, nonwaxy)	
Single crossing over (sh-Wx)	C sh wx	C sh wx/c sh wx (coloured, shrunken, waxy)	1,227
	c Sh Wx	c Sh Wx/c sh wx (colourless, full, nonwaxy)	
Double crossing over (C-sh) (sh-Wx)	C Sh Wx	C Sh Wx/c sh wx (coloured, full, nonwaxy)	6
	c sh wx	c sh wx/c sh wx (colourless, shrunken, waxy)	

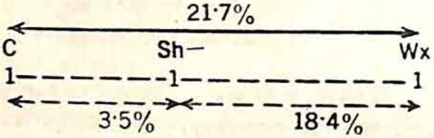
According to data—

$$\text{Recombination } C-sh=\frac{229+6}{6708}\times 100=3{\cdot}5\%$$

$$\text{Recombination } sh-Wx=\frac{1227+6}{6708}\times 100=18{\cdot}4\%$$

$$\text{Recombination } C-Wx=\frac{229+1227}{6708}\times 100=21{\cdot}7\%$$

The recombination value $C-Wx$ (21·7%) is close to $C-sh+sh-Wx=3{\cdot}5+18{\cdot}4=21{\cdot}9\%$ and therefore *Sh* should be located between *C* and *Wx*. The linkage map will be



Based on different findings several chromosomal maps have been prepared. In case of *Drosophila*, Bridges discovered that no crossing occurred in male individuals and therefore male *Drosophila* is an example of complete linkage. However, there is no suitable explanation to account for such a concept in this case. Similarly, Emerson prepared the chromosome map of maize chromosome and location of several hundred genes are now known.

Cytological basis of crossing over

In the breeding experiments, the failure in law of independent assortment shows linkage and formation of recombination to crossing over. Since the homologous chromosomes normally exchange reciprocal segments, hardly the progeny will show any morphological difference. Therefore, the presence of chiasmata remains the only cytological evidence of crossing which is not necessarily to be associated with exchange of chromosome segments. Some experiments have been specially devised to demonstrate crossing over.

STERN'S EXPERIMENT IN *Drosophila*—Stern (1931) selected female *Drosophila* which carries a translocated small part of *Y*-chromosome on its one *X*-chromosome. This *X*-chromosome was normal (+ +). The other *X*-chromosome of fly was partly broken and carrying mutant alleles for carnation eye 'car' (darkish red eyes, recessive) and barred eye 'B' (narrow eyes, dominant). So the female fly was heterozygous for both these genes (car B/+ +) and had barred eyes (since car is recessive to +). Both these *X*-chromosomes can be distinguished cytologically. Hutchinson made a cross between such a female and male fly having recessive alleles for both these genes (car, +). The results are shown in the Fig. 6.9.

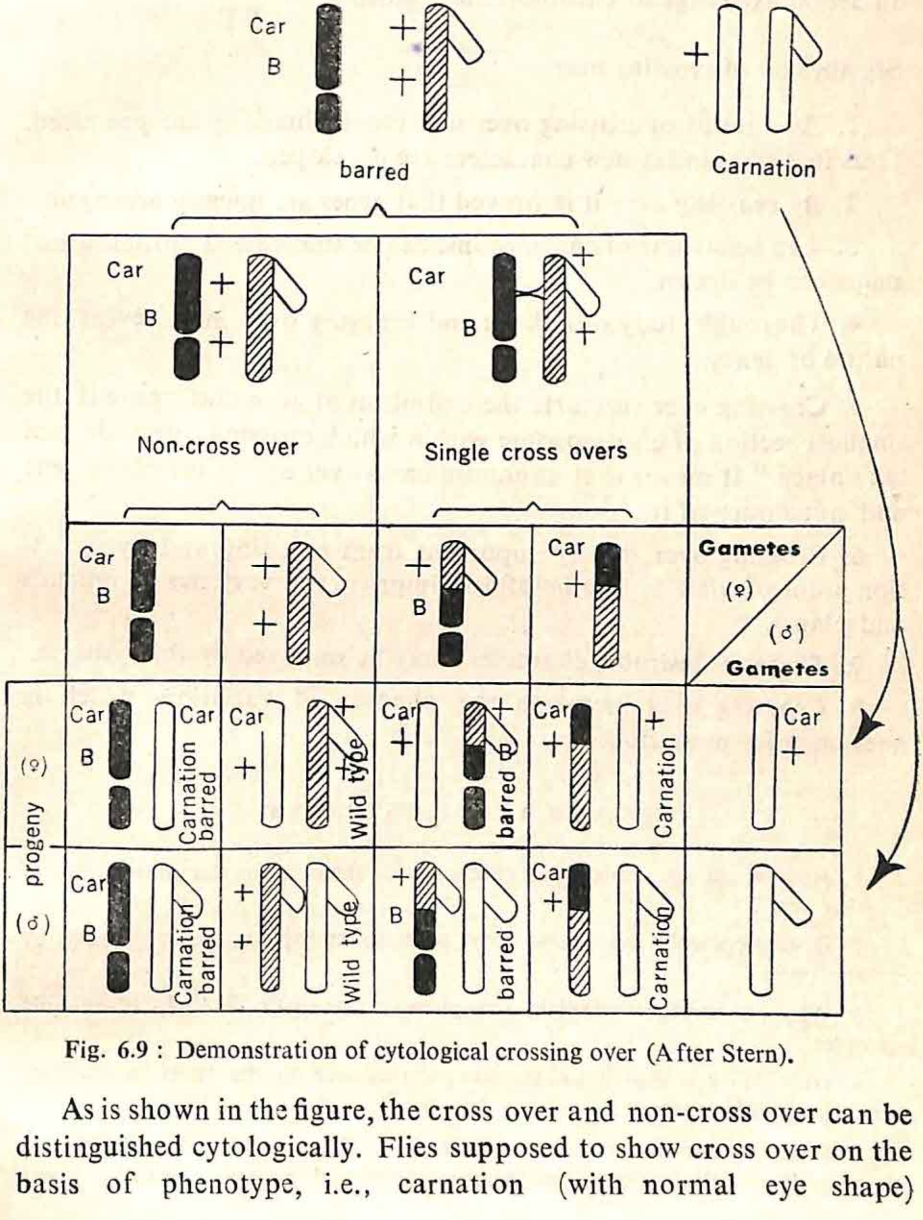


Fig. 6.9 : Demonstration of cytological crossing over (After Stern).

As is shown in the figure, the cross over and non-cross over can be distinguished cytologically. Flies supposed to show cross over on the basis of phenotype, i.e., carnation (with normal eye shape) and barred (with normal eye · colour) were studied cytologically. Carnation flies did not contain any fragmented *X*-chromosome rather it had normal *X*-chromosome. While barred flies had a fragmented *X*-chromosome with a *Y*-segment attached to one of the two fragments of *X*-chromosome. These cytological investigations suggest that genetic cross over was accompanied with an actual exchange of chromosome segments.

Significance of crossing over

1. As a result of crossing over new recombinations are produced. Thus in an organism new characters are developed.

2. By crossing over it is proved that genes are linearly arranged.

3. The behaviour of chromosome can be studied and chromosomal maps can be drawn.

4. Thorough study of linkage and crossing over may reveal the nature of genes.

5. Crossing over supports the definition of gene that "gene is the smallest section of chromosome within which crossing overs do not take place." It means that minimum cross over unit is the whole gene and not a part of it.

6. Crossing over is very important from breeding and hybridisation point of view as it is helpful to improve the varieties of animals and plants.

7. The non-desirable characters may be removed by this process.

8. Crossing over increases the chances of variation which is necessary for evolution.

GENERAL QUESTIONS

1. How would you explain that chiasmata are the cause or the consequence of crossing over ?

2. Discuss critically the various views proposed to explain the mechanism of crossing over.

3. What is linkage ? Explain the phenomenon with the help of suitable examples.

4. How will you explain linkage and crossing over on the basis of chromosome theory of inheritance ?

5. From a three-point test cross (*CshWx/cShwx* × *cshwx/cShwx*) with the following data, calculate the recombination values and prepare a relative linear

linkage map showing position of genes.

$CshWx=2777$ $CShwx=116$
$cShwx=2708$ $cshWx=123$
$Cshwx=643$ $CShWx=4$
$cShWx=626$ $Cshwx=3$ **Total**$=7000$.

6. Construct a map of the chromosome showing relative position of gene from the following data.

$r-c=10$ units $S-c=18$ units
$c-P=13$ units $S-r=8$ units.
$P-r=3$ units

* * *

CHAPTER 7

CYTOLOGY IN RELATION TO TAXONOMY

In taxonomy, the main problem centres around arranging plant and animals into a standard systematic units, e.g., Order, Families, Genera, Species etc. Earlier this problem was tackled on the basis of evaluating external resemblances between them. Modern taxonomy is quite advanced and much more than this. Many disciplines have been included to improve the aspect of finding out true relationships between the organisms. Of these, cytology has found a new field of usefulness in taxonomy. Many cytological characters such as number, morphology and behaviour of chromosomes have been employed in determining the true relationships of the organisms. In the first step, cytotaxonomy has been used in exploring the cytological relationship between the natural taxonomic units but it is also now used in finding out the varied effects of alterations in the chromosome complements to ascertain wide conception of the origin of the diversities observed in the living world.

Plants have proved better tools, therefore, more extensively used in persuing cytotaxonomical studies. It may be due to occurrence of polyploidy more commonly in plants than animals. Some aspects of cytotaxonomy are considered here.

Chromosome number

Though extensive studies have been done during last several years for determining the number of chromsomes per nucleus of hundreds of both plants and animals species, there is still a lot of work to be done to complete the list. Furthermore the reliability in number of chromosomes also varies. In many cases, the counts made are variable. However, careful observations are suggested to be taken up.

Many examples in plants have shown a clear cut numerical relationship, e.g., the genus *Nymphaea* with the species *stellata*, *lotus*, *odorata*, *candida* and *gigantea* have somatic numbers 28, 56, 84, 112, 224, respectively. On this basis, it can be visualised that the basic number for the genus would be 7 but no case of somatic number of 14 has yet been recorded. *Plantago* has given another trend in which three categories of basic number has been recognised (i) plants only diploid with 6 basic number, (ii) plants both diploid and tetraploids with this basic number and types with a lower basic number and (iii) plants containing polyploid species with basic number 6, 5, 4 and 9 as derivatives. Likewise in *Triticum* (wheats), the species of einkorn group have 14 somatic chromosome, those of emmer group 28 and spelta group 42. The wheat of spelta group is quite common.

The relationship in the chromosome number in the related genera of same family has been revealed in many cases. Sometimes, even their numbers are same. In Ericaceae, at least ten of genera show one or more number of species, 6, 12, 18, 24, 36 and 48 with 24 as the commonest somatic number. Also in Malvaceae, as many as five polyploid series are known represented with 5, 6, 7, 11 and 13 as the basic numbers. In Leguminosae, the polyploidy is uncommon. A. K. Sarkar worked on genus *Primula* and found $2n=22$ in *P. scapigera*, *P. obconica*, *P. khasiana*, *P. sinensis*, *P. prolifera*, *P. polyantha* and *P. tenella*. Similarly G. N. Bhattacharyya worked on Araceae and observed that $2n=28$ in *Arisaema utile*, *A. erubescens*, *A. decipiens*, *A. concinnum*, *A. griffithii*, *A. speciosum* while $2n=26$ in *A. tortuosum*, *A. sikkimense*, *A. nepenthoides* and *A. wallichianum*.

It has been found that above family level, the number of chromosomes as criteria for usage in cytotaxonomy in establishing the relationship is of meagre value. Even then certain points are worth and pertinent, e.g., most fungi have very low chromosome number, true ferns have high number, plants of liliaceae and amphibians have large chromosomes. However, it can be concluded that the number, size and form of chromosomes are useful but would not be of that value as the kinds and assortment of genes in major evolutionary lines may be.

Chromosome morphology

Like chromosome number, the morphology has also been considered in cytotaxonomy. In many cases, particularly those plants and animals which have very small and numerous chromosomes, it is humanly impossible to entertain such characters in the study of relationship. However, morphological characters can be more easily visualised in the organism which have larger chromosomes.

Crepis is the best example which has proved asset to the cytologists for the reason that the chromosomes are morphologically well differentiated and genomes being composed of few numbers.

The chromosome morphology has played determining role in establishing generic relationships among the members of Ranunculaceae. The results are quite close to arrangement as suggested by Engler system, but a few modification, based on chromosome morphology has been suggested by Gregory. He suggested that tribes such as Thalictraceae and Coptideae arose separately and not from the common stalk of Helleboreae and Anemoneae. Engler placed *Ranunculus* at top while cytological observation suggested that *Anemone* should be kept at top following *Clematis* in the same tribe.

Chromosome behaviour in crosses

Similarity in the chromosome behaviour during crosses offers an evidence of special value for exploring relationships among organisms. The two forms which may or may not be quite similar in appearance, if show sufficient similarities in behaviour of chromosome during crosses, extend a strong degree of evidence of relationship between each other.

During meiosis, the synapsis occurs in between homologous chromosomes which is considered as a critical example of genic similarity among chromosomes concerned. Any lack of synapsis, therefore, indicates a lack of close relationship between them. In diploid hybrids, there may be perfect success or total failure in synapsis and accordingly fertility varies with the degree of success. Longley managed a cross to produce triple hybrid from *Zea* and related genera, i.e., a cross in between *Zea*, *Euchlaena* and *Tripsacum*. The results were quite interesting. The *Zea* and *Euchlaena* chromosomes pair leaving the *Tripsacum* chromosomes unpaired except for an occasional trivalent. Thus two genera are closely related. These findings are quite valuable and helpful in establishing generic relationships.

A further sophisticated study has been made on *Drosophila* where it has been possible to make the most minute comparisons of chromosomes of related species because of giant chromosomes in their salivary gland chromosomes. Similar studies were made on certain geographical strains of *D.pseudoobscura* and it was found that in the III chromosome three different ways of results of inversion were present; e.g., (i) ABCDEFGHI, (ii) AFEDCBGHI and (iii) AFEHG BCDI. It can be concluded that due to successive inversions, their origin may be traced back and sequence may be (1)→(2)→(3) or (3)→(2)→(1) or it is possible that (2) might have been precursor of both (1) and (3)

CONCLUDING REMARKS

In many cases, Cytology has shown a poor face in solving the problems to establish the natural relationships between the organisms and it appears as if this branch is of no more importance in concluding the taxonomical disputes, yet whenever, they prove valuable, they do compensate well for the efforts expended upon them. In quite many cases, Cytology has enabled workers to decide whether an organism type newly observed in the field is a Mendelian variant, a heteroploid derivative of a familiar species or a stable and fertile interspecific hybrid.

Cytology's another important attribute to taxonomy has been to offer clues to resolve the problem on origin of species and other taxonomic units and thereby evolution. To the several factors as proposed by Darwin, Lamarck and others, Cytology provides the inner cause of variations.

In India, A. K. Sharma has contributed a lot in the field of cytotaxonomy. He has earned several awards for his valuable work and is a scientist of international repute. Under his guidance, the research work on varied branches is being done. Cytotaxonomy has been one of the main lines of investigation undertaken in his laboratory. In number of cases, meiotic studies have been carried out to visualise the interrelationships and the lines of evolution within the taxa. Sharma (1956) proposed theory of speciation in asexually reproducing plants.

Cytotaxonomic studies in respect to evolution have been made in both monocotyledons and dicotyledons. It is not possible to summarise his contribution in present text, but a few tit bits are elucidated here. In **Liliales**, the tribe Scilleae show speciation due to the major role played by numerical variations of chromosomes. The cause of variation in the tribe Tulipeae and Aloineae has been the minute structual alterations in the chromosomes. In the tribe Polygonatae, several genera such as *Polygonatum sibiricum*, *Smilacina fusca*, *Clintonia alpina*, *Disporum cantoniense* etc. resemble each other in their chromosome morphology.

Cytotaxonomic studies on **Palmales** have been made by S. K. Sarkar who concluded that Palmae, as a group, is a homogenous one. The main chromosome numbers reported are $2n=28$ and 36 and two or more lines of evolution can be seen in several tribes. While working on **Cyperales**, B. Sanyal suggested that structural alterations accompanied by extensive polyploidy and aneuploidy have been responsible for the origin of species in this group. R. Mallick (1966) studied cytotaxonomy of **Polygonales.** He worked out 14 species of *Rumex* and found $n=10$ chromosomes in all these forms. He suggested that basic number, aneuploidy and structural alterations of chromosomes have been responsible for speciation in this order.

Besides, a handsome amount of work has been done on the line of cytotaxonomy and evolution particularly on aquatic angiosperms, monocotyledons, helobiae and several others by A. K. Sharma and his associates.

GENERAL QUESTIONS

1. Write an essay on "Cytology in relation to taxonomy".
2. Discuss critically that the chromosomes at various places of taxonomic confusion have solved many problems.

■ ■ ■

CHAPTER 8

MENDEL'S LAWS OF INHERITANCE

MENDELISM

The actual importance of Mendelism after Mendel (1865), who is recognised as 'Father of Heredity', was realised with the onset of twentieth century. Between 1900 and 1934 alone the publication of 10,155 papers on the subject indicates the extent to which Mendelism became vital, popular and important during that time.

Life of Mendel

Gregor Johann Mendel was a monk in the Augustanian monastery at Brunn in Austria (now Berne in Czekoslovakia). He was born in 1822 in Moravia. His ancestors were gardners. In 1849, for a year he worked on a temporary post in a school after which he had courses in science and mathematics at the University of Vienna, Austria. This training enabled him to learn how to design and analyse experimental work on heredity.

He started teaching work again in 1854 which he continued for fourteen years. During this period the famous garden pea experiments were carried out by him and the results were published in a monographic form in 1865. After the publication of this monograph unfortunately he could not devote much of his time to experimental work as he got engrossed in his administrative duties as head of monastery.

Pre-Mendelian era

The experiments on hybridisation to explain the cause of heredity were carried out long before Mendel. From the time of Aristotle to the eighteenth century, it was believed that in an offspring, the mother contributes the inert matter while father imparts the motion of life. However, Leonardo de Vinci (1452-1519) visualised that both male and female parents contribute equally to the heredity of the offspring. Most of such experiments on hybridisation were restricted to the economically important plants. The pioneer worker of the time was Joseph Kolreuter (1733-1806), a German botanist. He crossed two species of tobacco and drew following conclusions:

(i) The hybrids are usually intermediate between their parents.

(ii) Both parents contribute equally to the hybrids.

His experiments were not uniform because hybrids from wild species were either partially or wholly sterile with better vegetative growth than parents. Soon the experimental accuracy was obtained by Knight (1759-1838) as regards segregation and expression of characters. Later, experiments on pea hybridisation were carried out by John Goss (1822). He selected two varieties of pea—blue and yellow and cross pollinated them. In the first generation, all pods were yellow. These hybrids were self pollinated and the pods which developed from such a cross were a mixture of yellow and blue peas. While repeating the experiments he concluded that plants arising from blue seeds produce blue peas only while those from yellow seeds were of two kinds—some producing a mixture of blue and yellow pods and others only yellow pods. Unfortunately he failed to visualise the exact significance of his findings.

Naudin (1862) while conducting experiments on plant hybridisation was a little ahead in concluding certain remarkable points as follows:

(i) A hydrid contains potentialities of both the parents.

(ii) At the time of formation of gametes, these potentialities segregate from each other.

(iii) The pattern of segregation applies to single potentialities or characteristics.

The last two points of Naudin were very close to the findings of Mendel and, thus, formed the basis of Mendel's laws. Unfortunately all these workers failed to give any numerical treatment to their results and therefore could not interpret them evidently. Later, only this fact became the secret of Mendel's success.

G. J. Mendel

Mendel was lucky in selecting the garden pea as his experimental material. He intelligently avoided all complexities that troubled earlier workers and then restricted his attention at a time only to a single trait such as height of the plant or colour of the flower. After establishing the exact behaviour of obtained hybrids, he advanced these experiments and considered two characters simultaneously.

Fig. 8.1 : G. J. Mendel

Mendel worked on pea for eight successive years (1857-1865) and presented his historical monographic work before the "Society for the Study of Natural Sciences." It was published in the Annual Proceedings of the Society, 1866. His original paper was entitled "*Versuche uber pflanzenhybriden* (experiments on plant hybridisation). Unfortunately these were the days of Darwin era when the entire world was engrossed in exploring the process of organic evolution. Mendel was therefore ignored. Besides, he faced utter frustration specially because of criticism given by Koelreuter, Gaertner and Hubert. But keeping confidence in his experimental findings he reiterated :

> "those who survey the work done in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of hybrid appears or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations."

Mendel died in 1884 unrecognised and bitterly disappointed. However, his great contribution was brought to light after 35 years by three botanists namely Hugo de Vries of Holland, Carl Correns of Germany and Von Tschermark of Austria. They independently rediscovered law of segregation proposed by Mendel. de Vries was first to unearth the Mendel's paper on law of segregation. Soon it had the support of Bateson in England, Castle and Devenport in U. S., and Cuevot in France. Now it was the continued era of Mendel.

Mendel's experiments

Mendel kept following points under consideration in his experiments on pea:

(i) Well definite plan of experiment.

(ii) Selection of plants with stable characters and with capacity to avoid cross pollination.

(iii) Plants and hybrids should not create difficulties in future process of hybridisation.

(iv) A careful record of various characters exhibited in the hybrids.

(v) Selection of healthy plants as weak plants show irregularities in the experiment.

(vi) Arrangement of reciprocal crosses in a way that if in first experiment pollen of one plant and egg of other are taken, vice versa should follow in next generation.

(vii) To raise and preserve the controlled plants with each experiment.

(viii) Statistical testing of experimental findings.

Mendel selected garden pea as his experimental material in view of the following advantages:

(i) plant has well defined characters and bisexual flowers.

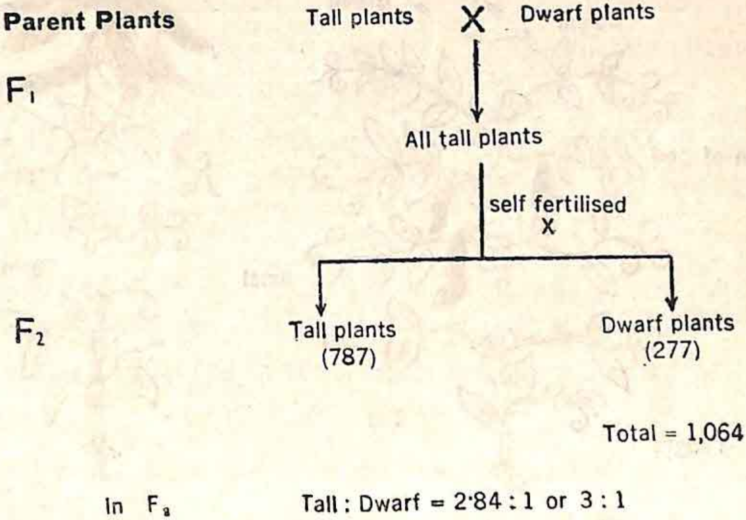
(ii) flowers predominantly show self fertilisation.

(iii) flowers are easy to handle during hybridisation.

(iv) plants do not occupy any large space and their flowering period is amicable.

He considered the presence of group of distinguishing characters in pea but he carefully recorded the behaviour of characters in unit form. Before the commencement of experiments he studied the stability and specificity of characters of the plant for several generations.

In his experiment, Mendel, first of all selected plants of different heights, one tall (about 45 cm. long) and one dwarf (about 22 cm. long) and made a cross between them. The seeds from these plants were taken and raised and their hybrids were measured. These raised hybrid plants were called plants of first filial generation (F_1). All F_1 plants were tall. The F_1 plants were self crossed and their F_2 were raised. In Mendel's experiment, about 1064 plants were produced in F_2. Of these, tall plants were 787 and dwarf 277 which means that tall and dwarf plants were produced in the ratio of 2·84 : 1. It can also be represented as follows :



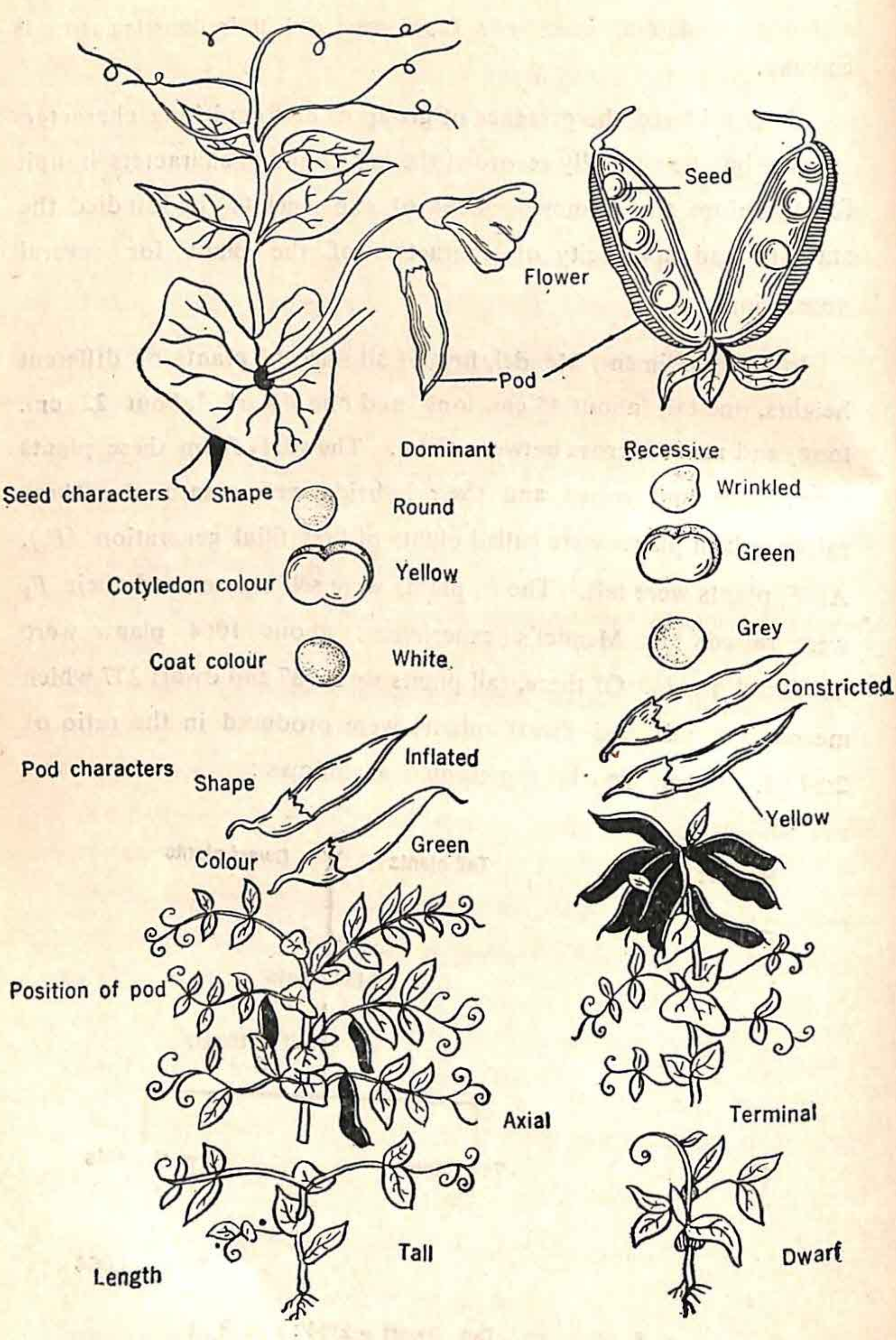


Fig. 8·2 : Some characters of Pea for hereditary experiments.

Mendel also made other experiments considering different characters and the findings of those experiments are given in table—8.1.

TABLE 8.1.

Serial no.	*Characteristics of Crosses*	*Dominant character expressed in F_1*	*Hybrid characters expressed in F_2*		*Ratio between dominant and recessive characters*
			Dominant character	*Recessive character*	
1.	**Kinds of seeds**				
	Round × wrinkled	Round	5474	1850	2·96 : 1
2.	**Colour of cotyledons**				
	Yellow × green	Yellow	6022	2001	3·01 : 1
3.	**Colour of flowers**				
	Red × white	Red	705	224	3·15 : 1
4.	**Shape of pods**				
	Inflated × constricted	Inflated	882	299	2·95 : 1
5.	**Colour of pods**				
	Green × yellow	Green	428	152	2·82 : 1
6.	**Position of flower**				
	Axial × apical	Axial	651	207	3·14 : 1
7.	**Height of the plant**				
	Tall × dwarf	Tall	787	277	2·84 : 1
	Total plants		14,949	5,010	2·98 : 1

Experimental findings on pea as regards different characters led Mendel to conclude following facts :

(i) Of the two contrasting characters, one of which is expressed in F_1 is called dominant character and other which does not appear is the recessive character.

(ii) The agencies carrying hereditary characters from generation to generation are called **factors** or **determiners** or **traits.** These traits are not destroyed in the hybrids and are expressed as such in the next generations.

(iii) These factors appear in the next generation in a proper ratio, i.e., 3 : 1.

In other experiments, Mendel considered two characters at a time and found that the distribution of factors or characters still maintain a definite ratio.

Terminology

DOMINANT CHARACTER : Of the two characters present in parents, one which is expressed in F_1 is called dominant character (it suppresses the presence of other character).

DOMINANT GENE : A gene that suppresses the action of another gene affecting the same feature (s).

RECESSIVE CHARACTERS : A character which is not expressed in F_1 but remains present in the hybrid. It appears in F_2.

ALLELES (ALLELOMORPH) : A pair of contrasting characters is called alleles. (Allel is the abbreviated form of allelomorph which means one form or other.)

PHENOTYPE : The sum of characters manifested by the organism. It may also be called as the visible characters of an organism with respect to one or more characters, e.g., red and white colour.

GENOTYPE : The genetic constitution of an organism which may not be apparent from the appearance.

HOMOZYGOUS : The organisms having identical genes, i.e., not having different allelomorph, is called homozygous. It may also be defined as that if the two chromosomes of a pair carry identical genes, the organism is called homozygous.

HETEROZYGOUS : Opposite to homozygous.

DIHYBRID CROSS : If in the experiment two characters are considered at a time, it is referred as a dihybrid cross.

FILIAL GENERATION (F) : It means either kind of offspring, i.e., male or female. F_1 is used for first filial generation and F_2, F_3 for second and third generations, respectively.

BACK CROSS : When F_1 hybrid is crossed with its either parents, it is called back cross.

TEST CROSS : When F_1 hybrid is crossed with its recessive parent, it is referred as test cross. So every test cross will be a back cross but every back cross will not be a test cross.

MENDEL'S LAWS OF INHERITANCE

Based on experimental findings on garden pea, Mendel proposed certain well established laws that are popularly called Mendel's laws of inheritance. These include two major ones—the segregation and the assortment of genes together with less important one—as the dominance and recessiveness of traits in the hybrids. These are further discussed here.

Law of Dominance (First law of Mendel)

Mendel crossed a true breeding tall plant (45 cm. long) with a true breeding dwarf plant (22 cm.) and found that all plants produced in F_1 (hybrids) were tall. He referred to tallness as a dominant character and dwarfness as a recessive character. Thus, dominant character is one which appears in F_1 whereas which did not appear is recessive.

Such an expression was found in the test of all the seven contrasting characters selected by Mendel. These results shown in table 8.I elucidate the fact that tallness is dominant over dwarfness, the round form of seed is dominant over the wrinkled, yellow colour of cotyledons over the green, the inflated pod over constricted one, the green colour of the ripen fruit over the yellow colour, axillary

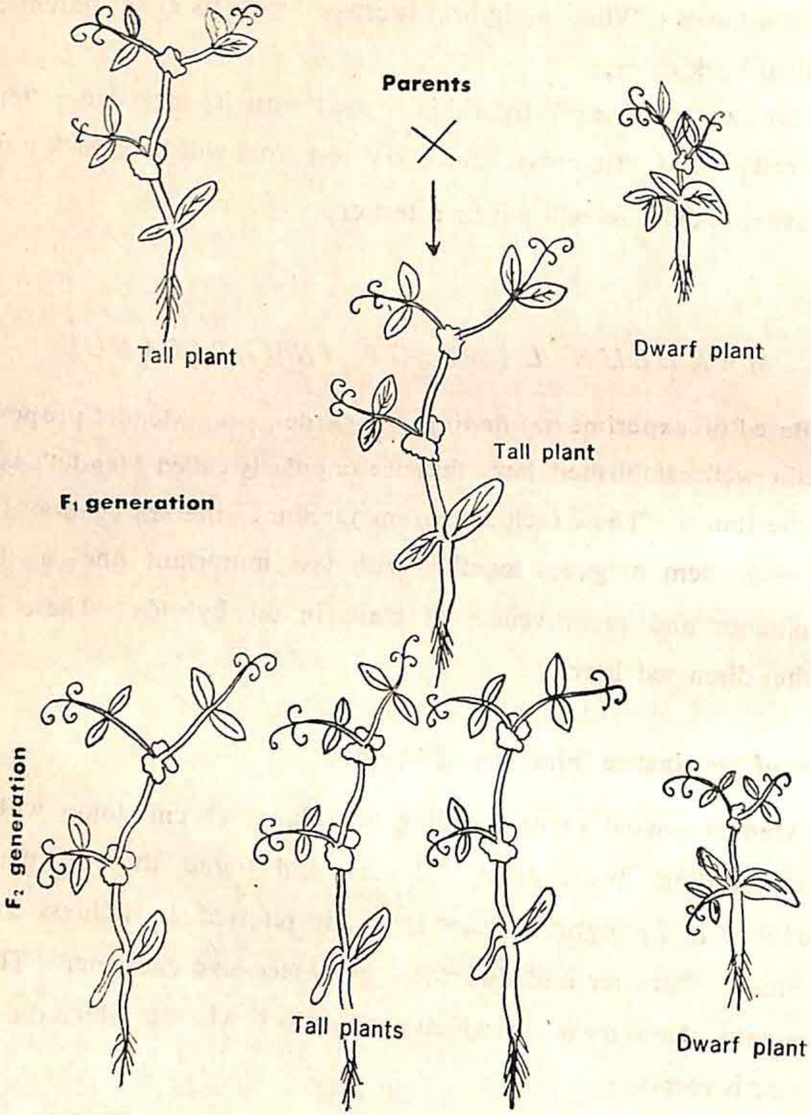


Fig. 8.3 : The findings of Mendel while conducting experiments on tall and dwarf varieties of Pea. The results are shown upto F_2 generation in which case tallness and dwarfness segregate in the ratio of 3 : 1. In F_1, only tallness appears.

position of flower over terminal position and red colour of the petal over white.

From these findings Mendel concluded that *in true breeding varieties, the first generation hybrids are all uniform with respect to*

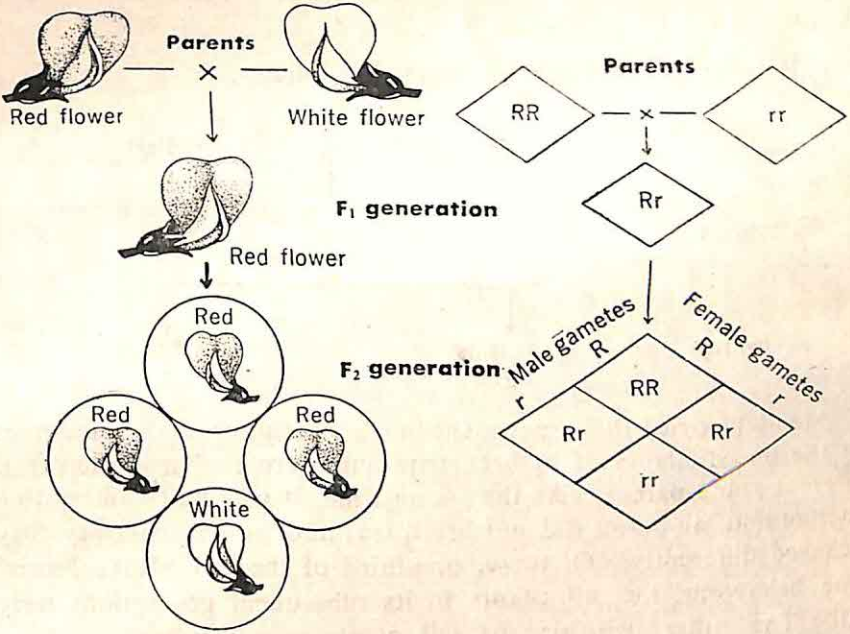


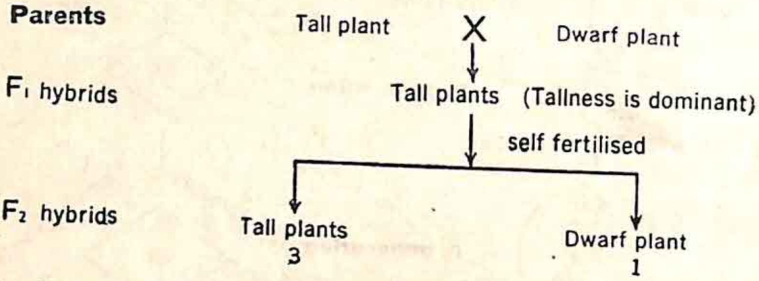
Fig. 8.4 : Experiments conducted by Mendel on Pea. When the cross between red and white flower was made, it was found that, in F_1 only red flowers appeared (dominant character) while in F_2 generation, the red and white colour segregated in the ratio of 3 :1. In the diagram both phenotypic (on the left side) and genotypic composition (on the right) are shown.

the trait in question and in case the dominance of a trait in one of the parents is complete, the hybrid will always resemble with that parent which carries the dominant trait. To this he referred "Law of dominance."

Law of Segregation (Second law of Mendel)

Mendel extended his experiment further. He managed self fertilisation among tall F_1 hybrids and thus obtained F_2 of the same experiment. He found two kinds of F_2 plants—the tall and the dwarf plants. Since these F_2 plants were indentical to their grandparents, he concluded that both these characters were present in F_1 but have segregated and expressed in F_2. Three-fourth plants of F_2 were tall and resembled to dominant grand-parent while one-fourth were dwarf and resembled the recessive grand parent, i.e., to say that the dominant and recessive character segregated again in the ratio of 3 : 1.

This cross can be further expressed as follows:



Mendel carried this experiment to F_3 generation and found that all the dwarf plants of F_2 bred true and were similar in their trait to great-grand-parent. At the same time, it was quite interesting to note that tall plants did not breed true and on the contrary they behaved differently. Of these, one-third of the tall plants showed true behaviour, i.e., all plants in its subsequent generations were tall. The other two-third of tall plants were heterozygous and behaved similar to F_1 plants, i.e., they produced two kinds of plants in the ratio of 3 tall and 1 dwarf.

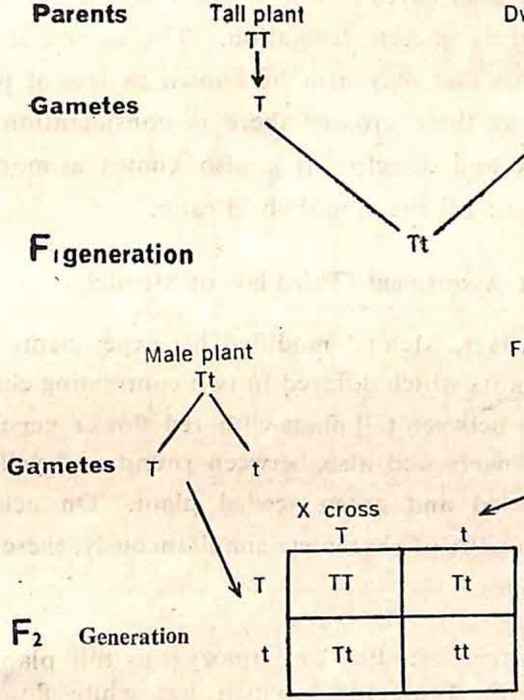
THE MECHANISM OF SEGREGATION : These findings of the experiment could be further illustrated to make the law still clear. It may be briefed as here.

An individual plant is produced as a result of the union of the male and female gametes. Mendel proposed that gametes do not carry the characters as such rather they have hereditary units called factors or determiners in them which are now called genes. The character like tallness is carried by one kind of trait and dwarfness by another. The factors for tallness and dwarfness form an allelomorphic pair and are alleles to each other. He proposed to use symbol of letters to represent these traits, e.g., *T* for tallness and *t* for dwarfness (capital *T* for dominant character and small *t* for recessive character).

The true breeding tall plant has two factors or genes—*TT* and dwarf plant—*tt*. The gametes formed from tall plant will have a factor *T* while from dwarf plant *t*. During fertilisation they combine and form F_1 hybrid as *Tt*. Since tallness dominates over dwarfness, the F_1 plants were tall.

During fertilisation of F_1 hybrids, each plant will produce two types of gametes, 50% with factor T and 50% with factor *t*. Thus, there will be two types of pollen or two types of egg. At the time of fertilisation, they will combine at random with the result of which three types of plants in F_2 can possibly be produced—Pure tall (*TT*), hybrid tall (*Tt*) and pure dwarf (*tt*).

These findings may be expressed as follows :



Tall (pure) = 1 (*TT*)
Tall (hybrid) = 2 (*Tt*)
Dwarf (pure) = 1 (*tt*)

Thus, tall and dwarf factors segregate in the ratio of 3 : 1.

This ratio is true to express its phenotypic ratio but genotypically it will be 1 : 2 : 1. The pure tall and the pure dwarf, are homozygous and they breed true whereas hybrid talls are heterozygous which in the next generation again show segregation ratio 3 : 1.

Mendel carried this type of study for every allelomorphic pair and reached the same conclusion as shown in table 8. 1.

Thus law of segregation states that *when an allelomorphic pair is brought together in a hybrid (F_1), the traits or genes remain together in it (hybrid) without blending or contaminating each other and they separate when the hybrid forms gametes.*

Another conclusion may be derived from these findings that gametes are pure and can carry only one of the two alternative genes or traits. F_1 individual carries both T and t which get separated from each other during gamete formation. The gamete may carry either T or t. This fact may also be known as law of purity of gametes. Besides, in these crosses there is consideration of one character at a time and therefore it is also known as monohybrid cross and the ratio 3 : 1 is the monohybrid ratio.

Law of Independent Assortment (Third law of Mendel)

In some other cases, Mendel modified his experiments on pea and selected the plants which differed in two contrasting characters. He managed crosses between tall plant with red flower versus dwarf plant with white flowers and also between round and yellow seed plant versus wrinkled and green seeded plant. On account of involvement of two pairs of characters simultaneously, these are also called dihybrid crosses.

Mendel crossed true breeding or homozygous tall plant which has red flowers with dwarf plant which has white flowers. F_1 hybrids were self fertilised and F_2 were raised. The F_2 plants were of four kinds as regards to phenotype—

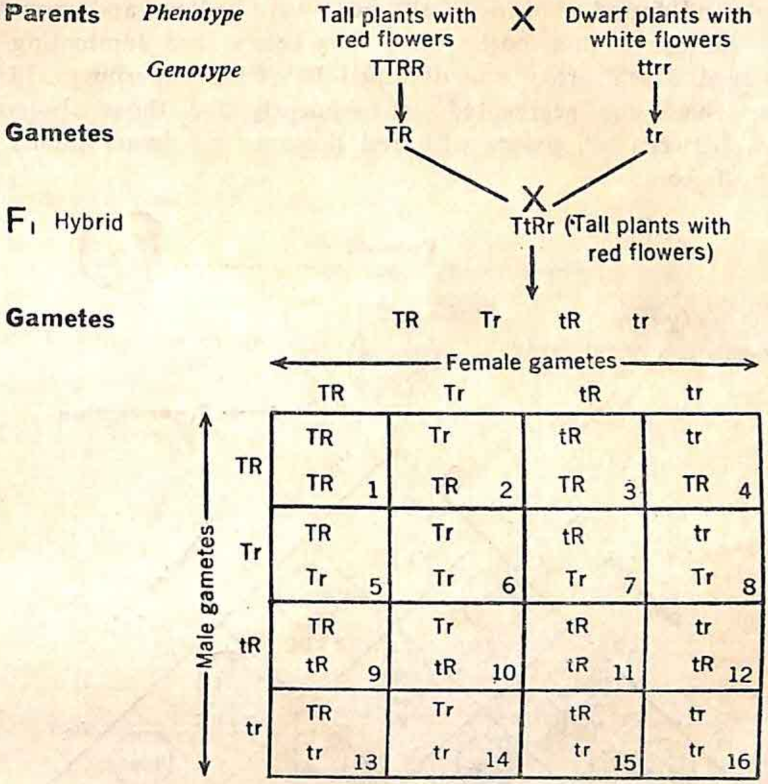
(i) tall plants with red flowers

(ii) tall plants with white flowers

(iii) dwarf plants with red flowers and

(iv) dwarf plants with white flowers.

These observations can be depicted in chart form as follows:



	TR	Tr	tR	tr
TR	TR TR 1	Tr TR 2	tR TR 3	tr TR 4
Tr	TR Tr 5	Tr Tr 6	tR Tr 7	tr Tr 8
tR	TR tR 9	Tr tR 10	tR tR 11	tr tR 12
tr	TR tr 13	Tr tr 14	tR tr 15	tr tr 16

Summary chart

No. of individuals with square no. of the chart	*Genotype number*	*Phenotype number*	*Pheno-type ratio*	*Breeding behaviour on self fertilisation*
1 (Sq. no. 1)	*TTRR*	Tall plants with red flowers	9	Breeds true for both traits
2 (Sq. nos.2,5)	*TTRr*			Breeds true for tallness
2 (Sq. nos. 3,9)	*TtRR*			Breeds true for red flowers
4 (Sq. nos. 4,7, 10 ,13)	*TtRr*			Heterozygous; segregates for both traits
1 (Sq. no. 6)	*Ttrr*	Tall plants with white flowers	3	Breeds true for both traits
2 (Sq. nos. 8,14)	*TTrr*			Breeds true for white flowers
1 (Sq. no. 11)	*ttRR*	Dwarf plants with red flowers	3	Breeds true for both traits
2 (Sq. nos. 12,15)	*ttRr*			Breeds true for dwarfness
1 (Sq. no. 16)	*ttrr*	Dwarf plants with white flowers		Breeds true for both traits

Mendel conducted similar type of experiment while making cross in between yellow and round seeded and green and wrinkled seeded plants and found that in F_1 all seeds were yellow and round. It thus indicated that both these characters are dominating. He managed a self cross and obtained F_2 of his experiment. In F_2, these characters segregated independently like those observed in cross between tall plants with red flowers and dwarf plants with white flowers.

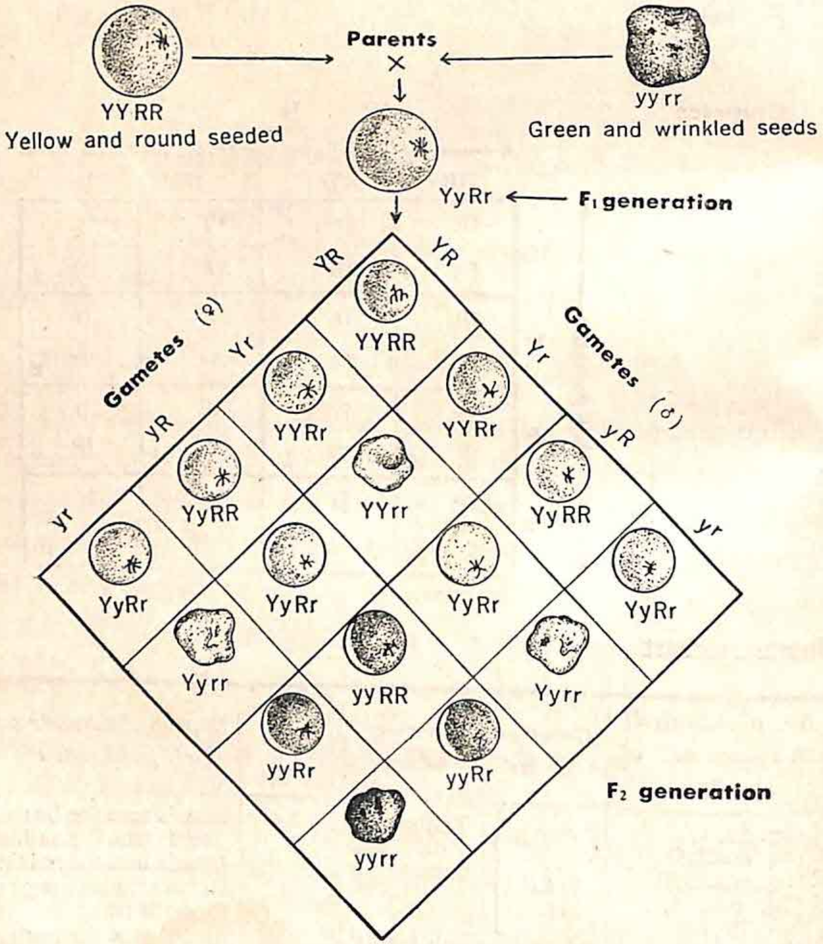


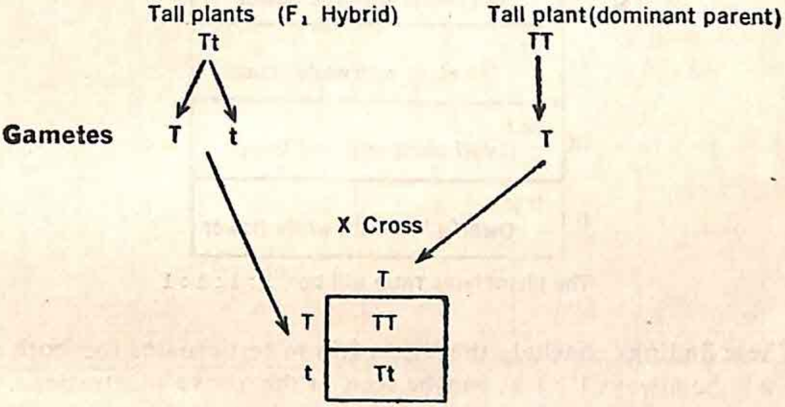
Fig. 8.5 : The results as observed in F_1 and F_2 from the cross between yellow and round seed & green and wrinkled seed. The seeds of F_1 plants were yellow and round seed and in F_2 the segregation ratio is 9 : 3 : 3 : 1 (9 yellow and, round seeds; 3 green and round seeds; 3 yellow and wrinkled and 1 green and wrinkled). The results are similar to those observed in cross between tall plants with red flowers and dwarf plants with white flowers.

This law concludes that *when two pairs of independent alleles are brought together in the hybrid (F_1), they at the time of gamete formation segregate or assort independently at random and freely.*

Law of independent assortment has been found applicable throughout the plant kingdom and also in animals such as guinea pigs, rats and rabbits. This law in certain cases has been regarded as second law of Mendel but when the law of dominance is taken into account, it is to be referred as third law of Mendel.

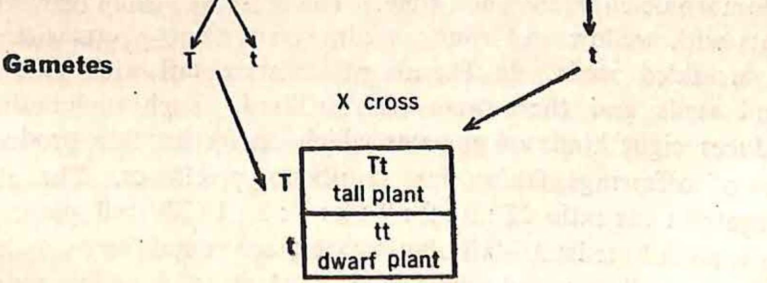
Back cross and Test cross

When one of the F_1 hybrids is crossed back with parent, it is called back cross. F_1 may be crossed either with dominant parent or with recessive parent. When F_1 individuals are crossed with dominant parents, the hybrids produced express only dominant character. All plants will have dominant character. It can also be expressed as follows:



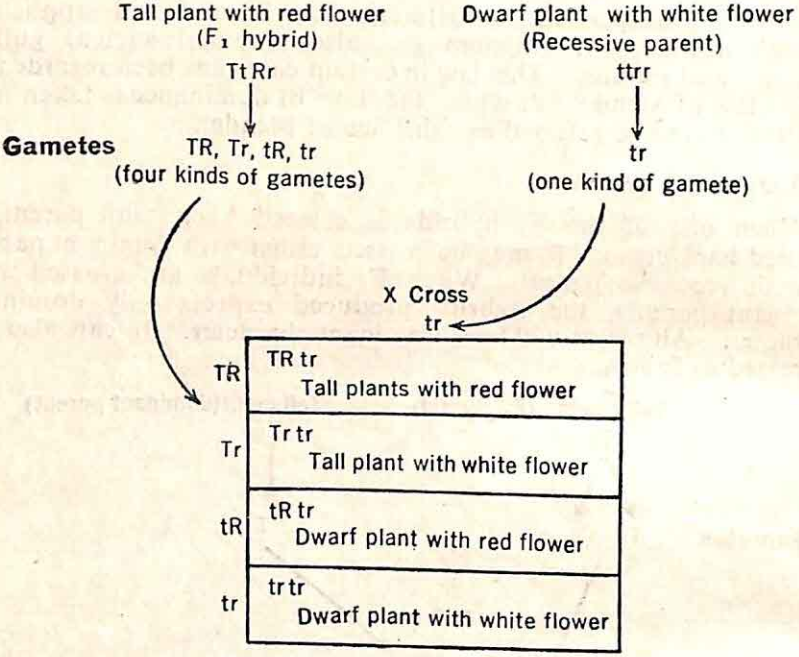
If the cross is in between F_1 individual and recessive parent, it is called *test cross*. Using test cross, the correctness of laws of segregation and independent assortment can be tested as follows:

(i) Test cross in law of segregation



The phenotypic ratio will be 1 tall : 1 dwarf.

(ii) Test cross for law of independent assortment


Tall plant with red flower
(F_1 hybrid)
TtRr
Dwarf plant with white flower
(Recessive parent)
ttrr
Gametes
TR, Tr, tR, tr
(four kinds of gametes)
tr
(one kind of gamete)
X Cross
tr
TR
TR tr
Tall plants with red flower
Tr
Tr tr
Tall plant with white flower
tR
tR tr
Dwarf plant with red flower
tr
tr tr
Dwarf plant with white flower

The phenotypic ratio will be 1 : 1 : 1 : 1

These findings conclude that the ratio in test crosses for both the laws will be always 1 : 1 as can be seen in the above illustrations. It reveals that if the segregation of classes is 1 : 1 in a test cross, both the Mendel's laws are correct in the sense that F_1 plants are hybrid and heterozygous.

Trihybrid and Polyhybrid crosses

Mendel extended his findings on pea considering the three pairs of allelomorphic characters at a time. The cross was made between tall plants with yellow and round seeds versus dwarf plants with green and wrinkled seeds. In F_1, all plants were tall with yellow and round seeds and these were self fertilised. Each such individual produces eight kinds of gametes which on fertilisation produce 64 types of offsprings falling into 8 phenotype classes. The classes segregate in the ratio 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (27—tall plants with yellow, round seeds; 9—tall plants with green round seeds; 9—dwarf plants with yellow round seeds; 9—tall plants with yellow wrinkled seeds; 3—dwarf plants with green round seeds; 3—dwarf plants with yellow, wrinkled seeds; 3—tall plants with green wrinkled seeds and 1—dwarf plant with green wrinkled seeds). The segregation between single pair of alleles again maintained the ratio 3 : 1, i. e.,

Tallness and dwarfness = 3 : 1 (48 : 16)
Yellow and green colour of seeds = 3 : 1 (48 : 16)
Round and wrinkled surface of seeds = 3 : 1 (48 : 16)

The results were in conformity with Mendel's numerical interpretations.

Mendel's laws were further visualised considering more than three characters at a time. The general findings are summarised in the following table:

No. of traits considered in the experiment	*No. of gametes formed in F_1 hybrid*	*No. of genotypes in F_2 if dominance is complete*	*No. of phenotypes in F_2 if dominance is complete*	*Ratio of possible combinations of F_1 gametes*	
1	2	3	2	4	Tested by Mendel
2	4	9	4	16	
3	8	27	8	64	
4	16	81	16	256	Calculated
5	32	243	32	1024	
6	64	729	64	4096	

PROBLEMS ON MENDELISM

Mendelian expectations can be theoretically calculated after understanding the basis of laws of heredity. Such methods of deducing the result of particular mating have served as a boon to the plant breeders who have done a lot in the crop and race improvements. There are many methods for calculating the Mendelian expectations. At the very first hand the students are supposed to determine the various types of gametes expected to be produced by each participating parent, e.g., homozygous parents form only one type of gametes whereas heterozygous parents more than one type. In a monohybrid cross, two types of gametes—one with dominant genome and other with recessive genome, in equal number are produced in a heterozygous parent while in double heterozygous four different kinds of gametes and in triple heterozygous eight different types of gametes are produced, i.e., each additional trait doubles the assortment of gametes. The following example may elucidate the fact :

Genotype of Parent	*Types of gametes*
Homozygous AA	all A
Double homozygous $AABB$	all AB
Triple homozygous $AABBCC$	all ABC
Triple homozygous $aaBBcc$	all aBc
Triple homozygous $aa\ bb\ cc$	all abc
Heterozygous $A\ a$	$A+a$ (Two types of gametes)
Heterozygous (double) $Aa\ Bb$	$AB+Ab+aB+ab$ (Four types of gametes)
Heterozygous (triple) $Aa\ Bb\ Cc$	$ABC+ABc+AbC+aBC$ $+Abc+aBc+abC+abc$ (Eight types of gametes)

Mendelian expectations may be calculated by one of the following two methods—Algebraic method or Punnet's checker board method.

Algebraic Method

In this method, the different types of gametes to be formed by either parent are determined which are then multiplied with those of other as in algebra. The product is the theoretical results to be expected among the offsprings. This fact can well be explained by taking the simplest example, i.e., a pure tall plant (TT) is crossed with a dwarf plant (tt). The gametes formed by the tall plant will be of one kind, all T type while those from dwarf plant will carry t.

The results of the mating of these two would be $T \times t$ or Tt and plant will be tall in F_1 generation. As a rule, F_1 are self fertilised to deduce the consequences in F_2. So is here. In F_1 two types of gametes $T+t$ will be formed. It is now calculated as follows :

$$\left(\begin{array}{r} (T+t) \\ \times\,(T+t) \\ \hline TT+Tt \\ Tt+tt \\ \hline TT+2Tt+tt \end{array}\right)$$

i.e., 25% pure tall (dominant)
50% hybrid tall
25% pure dwarf (recessive)
or 3 Tall : 1 dwarf (phenotype ratio).

If the cross is made between F_1 and the recessive parent (test cross), the following results would be expected.

Gametes of $F_1 = T + t$

Gamete of recessive parent $= t$

So

$$\begin{array}{l} T+t \\ \underline{\times t} \\ Tt+tt \end{array}$$

i.e., 50% hybrid tall plants

50% dwarf plants

or 1 Tall : 1 dwarf (phenotype ratio).

While applying algebraic method in a dihybrid cross, the following will be the calculation method and result.

Parents—Tall plant with red flowers (*TTRR*)

Dwarf plant with white flowers (*ttrr*)

Calculation—F_1 will be *TtRr* (Tall plant with red flowers dominant character)

Self fertilisation—$TtRr \times TtRr$

Applying algebraic method first work out the results for each trait separately.

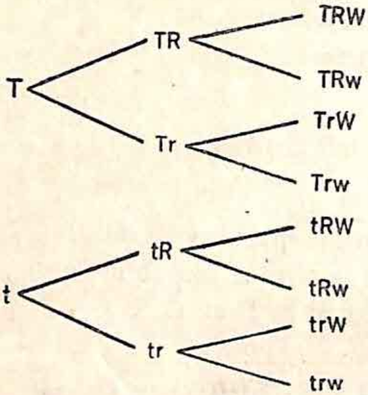
$$\begin{array}{c} T+t \\ \underline{T+t} \\ TT+2Tt+tt \end{array} \qquad \begin{array}{c} R+r \\ \underline{R+r} \\ RR+2Rr+rr \end{array}$$

The products are multiplied.

$$\begin{array}{c} TT+2Tt+tt \\ \underline{\times RR+2Rr+rr} \\ 1TTRR+2TTRr+1TTrr+2TtRR+4TtRr+2Ttrr+1ttRR+2ttRr \\ +1ttrr \end{array}$$

The result reveals that four different phenotypic classes will be formed in the cross as in a Dihybrid cross. This method becomes further complicated when more than two traits are considered simultaneously, therefore, it is better to adopt the other simpler method. One of them is the Punnet's checker board method—which is the same as studied under dihybrid cross—(law of independent assortment) as given by Mendel. The only difficulty that a student faces is in the formation of gametes when more than two traits are considered at a time. However, branching or forked-line method is the simplest one for deducing the various types of gametes to be produced by the parent. It should be followed. Once the gametes are formed, the checker boards are easy to make to derive the theoretical results.

Forked-Line Method for Deriving gametes



In this case one character is considered at a time and the two gametes are placed in separate forks. Then with each fork the gametes for second trait are taken into account. The same sequence is followed for any more number of traits to be considered. It can be further explained as follows :

Suppose the gametes from parent with *Tt Rr Ww* genotype are to be derived, the method is given on left side.

Eight types of gametes will be produced in a trihybrid cross. These gametes are placed in Punnet's Checker Board and the results are then calculated.

DEDUCING GENOTYPES

Its another type of Mendelian problem where in case of known results the genotypes of parents are calculated. Besides, the probable characters of the additional offspring resulting from such crosses may also be calculated. Some of the problems in this regard are discussed here.

Example 1 : In rabbits, black skin colour (*C*) is dominant to white skin colour (*c*). Two black rabbits when mated beget a white skinned rabbit. What would be the genotypes of the parents ?

Solution : White skin colour is a recessive trait in rabbits and when an offsping contains it, it should possess homozygous genome for white skin colour, i.e., *bb*. Since both parent rabbits were black and offspring white, the gene for white skin must be present in parents. If we presume that one parent is heterozygous (*Bb*) and other homozygous (*BB*), it will not be correct as the offspring will always be black (*BB* or *Bb*). If both parents are considered heterozygous, there will be a possibility of coming two recessive genes in the offspring which will be white in colour, e.g., parents contain heterozygous genome (*Bb*), the new offspring will contain either one of *BB*, *Bb* or *bb* genome. According to Mendel's law, if two heterozygous parents are mated, the produced offspring will be in ratio $BB+2Bb+bb$. So in the above cross it happened that two recessive genes came in one offspring which is possible only when both the parents are heterozygous (Bb & Bb).

Example 2 : In a cross between tall plant with round seeds and tall plant with wrinkled seeds produced a drawf plant with wrinkled seeds. Deduce the genotype when tallness (T) is dominant over dwarfness (t) while round seeds (R) over wrinkled seeds (r).

Solution : The offspring is dwarf plant with wrinkled seeds. Both these characters are recessive and are expressed in homozygous recessive form ($tt\ rr$). It is possible only when both parents contain these recessive genes and accidentlly during mating, the recessive genes come in the offspring which is a dwarf plant with wrinkled seeds. The example is further illustrated by taking the genotype of heterozygous parents. Genome of heterozygous parents will be—tall plant with round seeds ($Tt\ Rr$) and tall plant with wrinkled seeds ($Tt\ rr$) and by forming the gametes, checker board is drawn. Four types of gametes (TR, Tr, tR, tr) are produced from the former parent whereas two types of gametes (Tr, tr) from the other parent. Eight offsprings will be theoretically produced of which one will be dwarf plant with wrinkled seeds.

Example 3 : A plant with yellow round seeds when crossed with the plant green wrinkled seeds, plants with yellow round, yellow wrinkled, green round and green wrinkled are produced in equal number. What will be the genotype of the parent ? (Yellow (Y) is dominant over green (y) and round (R) over wrinkled (r).

Solution : Its an example of test cross as the ratio between four types of offsprings is 1 : 1 : 1 : 1 and in such case one parent is heterozygous ($Yy\ Rr$) i.e., plant with yellow round seeds and other parent is homozygous recessive ($yy\ rr$). Now it can be easily solved following the method of test cross described earlier.

Example 4 : In tomato red colour (R) and spherical shape of fruit (S) are dominant over yellow colour (r) and cylindrical shape (s). In a cross between red spherical and red cylindrical, the offsprings produced were 122 red spherical, 120 red cylindrical, 41 yellow spherical and 39 yellow cylindrical. Determine the genotypes of parents.

Solution : In the cross total number of produced offsprings are 322 which segregate in the phenotype ratio 3 Red spherical : 3 Red cylindrical : 1 yellow spherical : 1 yellow cylindrical.

Take one character at a time. In a cross both parents have red flesh colour of the fruit but in offsprings yellow colour also appears. Yellow colour can appear only when the offspring is homozygous for recessive characters (*rr*). It would mean that both parents would be heterozygous for red (*Rr*) colour of the fruit. Because only then recessive homozygous offspring can be produced.

Now take the second character, i.e., shape of the fruit. The cross was in between parents one with cylindrical shape (recessive character) and other with spherical shape (dominant character). The parent with cylindrical shape of fruit will be homozygous recessive (*ss*) only then the recessive character could be expressed. The parent with spherical fruit will be heterozygous which will form two types of gametes—one with dominant character (*S*) and other with recessive character (*s*). This parent is evaluated on the basis of producing offsprings showing the expression of recessive character, i.e., cylindrical fruit.

Hence the genotype of two parents will be *Rr Ss* for parent showing red and spherical fruits—and *Rr ss* for parent showing red and cylindrical fruits. This presumption can be confirmed on the basis of theoretical calculations as follows:

Parents	Red & Spherical RrSs ↓	X	Red & Cylindrical Rrss ↓
Gametes	RS, Rs, rS, rs (Four types)		Rs, rs (Two types)
Crosses	Shown in checker board.		

Gametes of red spherical parent

Gametes of red, cylindrical	RS	Rs	rS	rs
Rs	RSRs 1	RsRs 2	rSRs 3	rsRs 4
rs	RSrs 5	Rsrs 6	rSrs 7	rsrs 8

Columns—1, 3, 5 show the offsprings with red and spherical.
Columns—2, 4, 6 show the offsprings with red and cylindrical.
Column —7 shows the offspring with yellow and spherical.
Column —8 shows the offspring with yellow and cylindrical.

Thus it will give the required ratio of 3 : 3 : 1 : 1.

These examples are of simple types which follow the Mendel's laws of inheritance.However, these laws are not universally applicable and there are many variations to them. Some of them are discussed in separate chapter entitled "Modifications of Mendel's laws" and few problems related to them are discussed here.

Example 1 : In a cross between plant with purple flower and with a white flower, 243 white and 162 purple flowered offsprings were produced. Its an example of sweet pea where genes *C* and *P* alone produce white flowers and when both are present purple colour of flower is produced. Give the genotype of the parents.

Solution : The offsprings produced show a segregation ratio of 5 : 3, i.e., minimum number of plants required are eight. Of these 5 are white colour and 3 are purple colour. As stated above when both *P* and *C* are present, flowers are purple, so its an example of complementary genes. Assuming that if one parent is homozygous for purple flower, the genotype will be *PPCC* and it will produce only one type of gamete—(*PC*). From such gametes all the offsprings produced will bear purple flower. Naturally no parent will be homozygous. Now if we presume that one parent that forms purple flower, as in the cross, is heterozygous (*PpCc*), four types of gametes will be produced (*PC*, *Pc*, *pC*, *pc*). Obviously, the second parent should produce only two types of gametes in order to produce minimum number of offsprings, i.e., 8. It is possible only when the second parent has genotype either *Ppcc* or *ppCc*. It can be further calculated that either of these when crossed with heterozygous purple flower producing parent can give the required product.

Example 2 : While working on maize breeding, a green maize plant when selfed produced 75 plants green and 5 plants white seedlings (lethal). It puzzled the student. How would you evaluate the genotype of parents and the mode of inheritance of the pigment ?

Solution : In the above experiment, the phenotypic segregation is nearly 15 : 1 (15 green and 1 white). So its a case of duplicate genes where two or more genes in different chromosome pairs have an identical effect on the expression of one character, i.e., the presence of single gene has same effect as more than one gene has. The number of offsprings produced 16 as minimum means its an example of dihybrid cross where the segregation ratio is 9 : 3 : 3 : 1. But first three classes merge into one in the above cross indicating the presence of duplicate genes and there should be two pairs of genes in different chromosome pair (because of dihybrid cross). Then the two pairs of genes will be $A_1A_1A_2A_2$ and their alleles as $a_1a_1a_2a_2$. The $a_1a_1a_2a_2$ represent no colour ultimately expressing lethal effect, therefore, the selfed parents must be heterozygous as they survive. The genotype of crossing parents will be $A_1a_1A_2a_2$, each will produce four types of gametes (A_1A_2, A_1a_2, a_1A_2, a_1a_2) and the number of offsprings will be 16 as minimum. Besides, no other combination of the $A_1A_1A_2A_2$ and their alleles $a_1a_1a_2a_2$ will give 16 plants as minimum. It can be further calculated and confirmed by putting the gametes in Punnet's checker board which will produce 15 green and 1 white plant. Thus the genotypes of both the parents will be $=A_1a_1A_2a_2$. Mode of pigment inheritance is governed by duplicate genes.

There can be several other examples of the same types.

1. In pea experiments on breeding, a tall plant was mated with a dwarf plant. The offsprings produced by such a cross were one half tall and one half dwarf. Deduce the genotype of the parent.

2. What gametes will be formed in the following crosses of pea plants ? Determine the phenotypic ratio of the offspring from each cross.

1. $TtRr \times ttrr$
2. $TtRr \times TtRR$
3. $YYrr \times yyRR$
4. $YyRR \times yyrr$
5. $TtRr \times TtRr$
6. $YyRr \times Yyrr$.

3. In a cross between yellow round seeds and yellow round seeds, 47 yellow round, 17 yellow wrinkled, 15 green round and 5 green wrinkled offsprings are produced. Calculate the genotypes of the parent.

4. In the experiments on chickens following results were obtained :

(i) Rose comb cock $A \times$ rose comb hen B produces rose combed chick and also a single combed chick.

(ii) Cock $A \times$ single comb hen C produces single combed chick.

(iii) Cock $A \times$ rose comb hen D produces single combed chick.

Determine the genotypes of cock A, hen B, hen C and hen D and deduce the offsprings' character expected from further mating [Rose comb (R) is dominant to single comb (r)].

5. A cross between yellow round and yellow wrinkled produces a green wrinkled offspring. Give the genotypes of the parents.

6. In Snapdragon, a plant hybrid for red (*R*) and white flowers (*r*) is pink (*Rr*). What would you expect the genotypic and phenotypic ratio in a cross between pink flowers and red flowers and another cross between pink flowers and white flowers ?

7. Brown eyes (*B*) are dominant over blue eyes (*b*) and brown hairs (*R*) over red hairs (*r*) in man. A man with brown eyes and red hairs married a woman with blue eyes and brown colour. They got two children one son with brown eyes and red hairs and other daughter with blue eyes and brown hairs. Find out the genotypes of parents and children.

8. The gene for white fruit colour (*W*) is epistatic to that for yellow (*Y*) which is dominant over green (*y*) in a plant. In a cross white plant was mated with a yellow. The progenies produced segregated in the ratio of 4 white, 3 yellow and 1 green. Give the genotypes of the parents.

GENERAL QUESTIONS

1. Write an essay on Mendel's laws of inheritance.
2. Write short notes on :
   (a) Law of segregation,
   (b) Test cross,
   (c) Law of independent assortment,
   (d) Dihybrid cross.

■ ■ ■

CHAPTER 9

MODIFICATIONS OF MENDEL'S LAWS

Mendel's laws of inheritance are not of universal application as they have not been found true in each and every case. Many exceptions are now known to them. Some of them are discussed here in short. They are called modifications of Mendel's laws and most of them are commonly referred as interaction of genes or interaction of factors.

Modification of F_1 Ratio

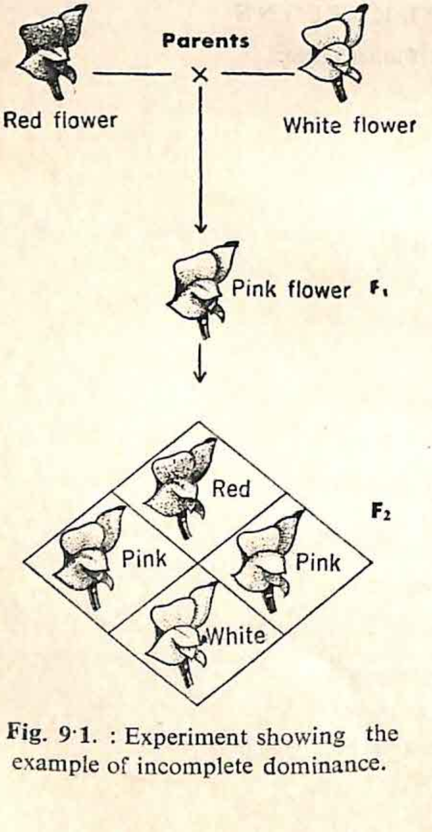


Fig. 9·1. : Experiment showing the example of incomplete dominance.

INCOMPLETE DOMINANCE : (Ratio—1 : 2 : 1)—In case of 4 o' clock plant (*Mirabilis jalapa*), a plant with red coloured flower was crossed with a white flowered one and was found that F_1 was pink (intermediate between two parents) and F_2 gave 1 red : 2 pink : 1 white.

Similar case has been recorded in rice when a cross between varieties T. 1083 with short outer glumes and E. B. 141 with long outer glumes is made. The F_1 appears intermediate between two parents in the size of the outer glumes and F_2 plants segregate into 1 short outer glume : 2 intermediate type : 1 long outer glume.

Both these examples challenge the universal occurrence of *law of dominance* and therefore, it becomes liable to be modified. It is now believed that the dominance may vary depending on various factors. Besides the occurrence of intermediate type as in *M. jalapa*, the examples of no dominance have also been found. In Snapdragon (*Antirrhinum*), however, the inheritance of petal colour serves as an example of incomplete dominance Fig. 9.1 while that of corolla shape of complete dominance.

Modifications of F_2 Ratios and Dominance

1. INTERACTION BETWEEN DOMINANT FACTORS : The examples of two pairs of factors pertaining to two different traits are quite common. However, there are certain examples, one discussed below, where two pairs of factors may affect the same traits. The phenotypic expression in such cases would naturally be the resultant of the action of the two genes.

Bateson and Punnett explained the inheritance of comb types in fowls. Four types of combs in fowl—(1) rose, (2) pea, (3) single and (4) walnut are known (fig. 9.2). Of these 'rose' is dominant over 'single' and symbolised as *R-r* and 'pea' is also dominant over single and symbolised as *P-p*. A cross is made between rose and pea and thus two dominant factors *R* and *P* are brought together. Both tend to produce comb of their own type and as a result of their combined action a new type *Walnut* is produced. It can be explained as

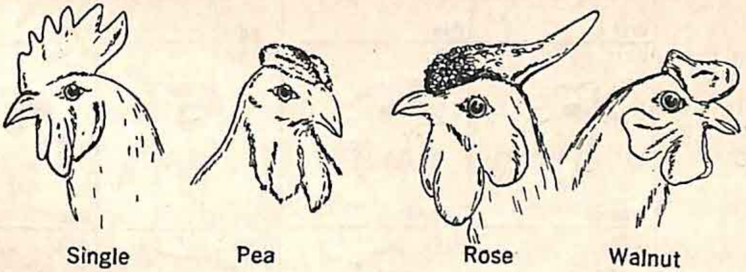


Fig. 9.2 : Different comb types in fowls.

follows:

Rose—*ppRR*
Pea —*PPrr*
Single—*pprr*

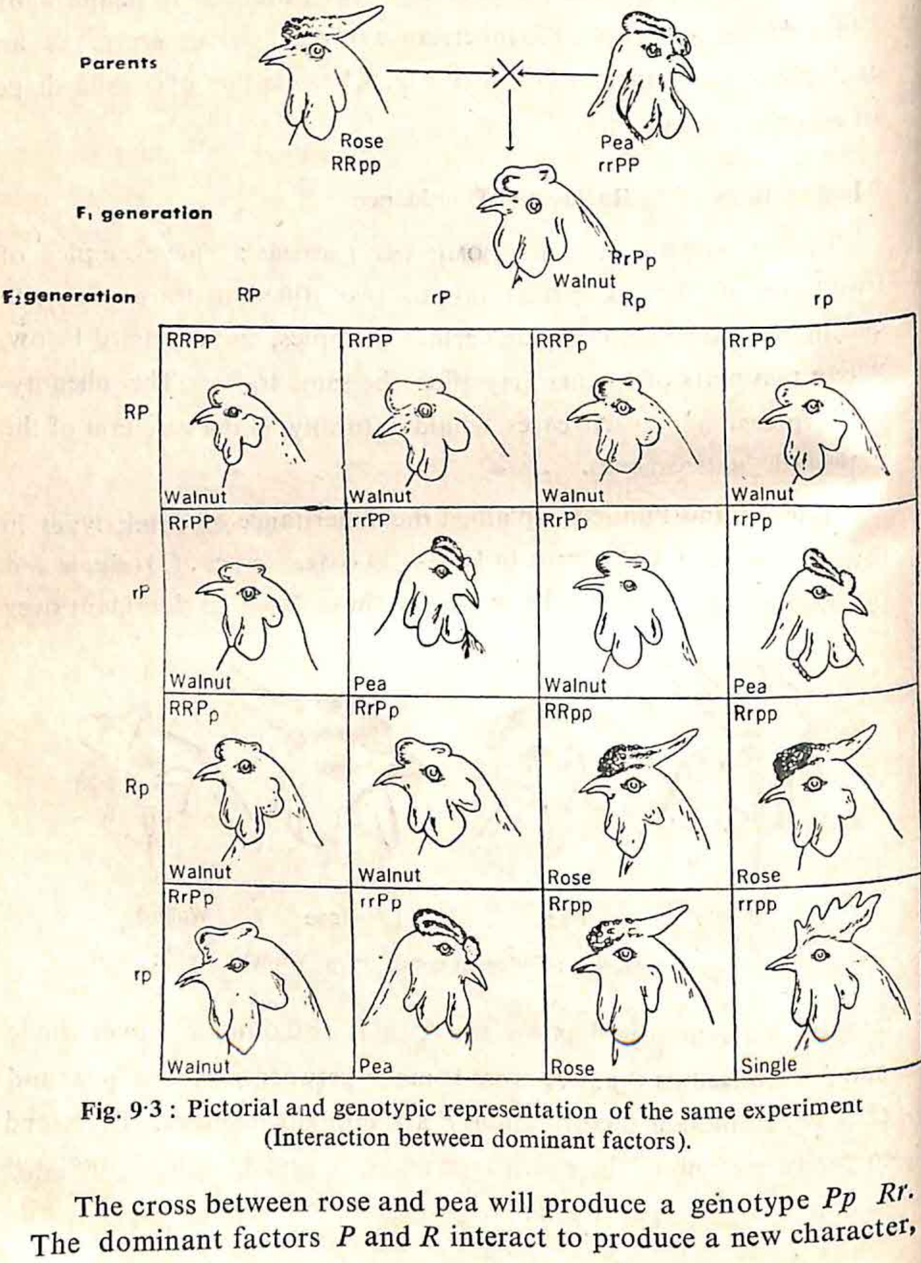


Fig. 9·3 : Pictorial and genotypic representation of the same experiment (Interaction between dominant factors).

The cross between rose and pea will produce a genotype *Pp Rr*. The dominant factors *P* and *R* interact to produce a new character, e.g., walnut type of comb. Both these factors would segregate in F_2 and data may be summarised as follows:

Table 9.1. Interaction between dominant factors

Phenotype	*Frequency*	*Genotype*	*Frequency*	*F_2 behaviour*
Walnut	9	*PPRR*	1	Breeds true
		PpRR	2	Segregates into 3 walnut : 1 pea
		PPRr	2	Segregates into 3 walnut : 1 rose
		PpRr	4	Segregates like F_1
Rose	3	*ppRR*	1	Breeds true
		ppRr	2	Segregates into 3 rose : 1 single
Pea	3	*PPrr*	1	Breeds true
		pprr	2	Segregates into 3 pea : 1 single
Single	1	*pprr*	1	Breeds true

Similar example has been observed in *Panicum miliaceum* where of the two factors four types of colours are produced.

2. COMPLEMENTARY FACTORS (Ratio 9:7). Two grey grained varieties of rice when crosssd resulted in F_1 plants with red grains and F_2 they segregated in an approximation to 9:7 proportion. This example is explained on the basis that two pairs of factors are involved in the interaction as follows:

Parents ⎡Grey grained type $= Pr_1\, Pr_1\, pr_2\, pr_2$
⎣Grey grained type $= pr_1\, pr_1\, Pr_2\, Pr_2$

F_1 hybrids — Red grains $= Pr_1\, pr_1\, Pr_2\, pr_2$

Gametes from $F_1 = Pr_1Pr_2$; Pr_1pr_2; pr_1Pr_2; pr_1pr_2.

The gametic combinations of F_2 are shown in the checker board.

		MALE GAMETES (♂)			
		Pr_1Pr_2	Pr_1pr_2	pr_1Pr_2	pr_1pr_2
FEMALE GAMETES (♀)	Pr_1Pr_2	Pr_1Pr_2 Pr_1Pr_2	Pr_1pr_2 Pr_1Pr_2	pr_1Pr_2 Pr_1Pr_2	pr_1pr_2 Pr_1Pr_2
	Pr_1pr_2	Pr_1Pr_2 Pr_1pr_2	Pr_1pr_2 Pr_1pr_2	pr_1Pr_2 Pr_1pr_2	pr_1pr_2 Pr_1pr_2
	pr_1Pr_2	Pr_1Pr_2 pr_1Pr_2	Pr_1pr_2 pr_1Pr_2	pr_1Pr_2 pr_1Pr_2	pr_1pr_2 pr_1Pr_2
	pr_1pr_2	Pr_1Pr_2 pr_1pr_2	Pr_1pr_2 pr_1pr_2	pr_1Pr_2 pr_1pr_2	pr_1pr_2 pr_1pr_2

The results may be summarised in following table.

Table 9.2. Complementary factors

Phenotype	*Frequency*	*Genotype*	*Frequency*	*F_2 behaviour*
Red	9	$Pr_1Pr_1Pr_2Pr_2$	1	Breeds true
		$Pr_1Pr_1Pr_2pr_2$	2	Segregates into 3 red : 1 grey
		$Pr_1pr_1Pr_2Pr_2$	2	Segregates into 3 red : 1 grey
		$Pr_1pr_1Pr_2pr_2$	4	Segregates like F_1
Grey	7	$Pr_1Pr_1pr_2pr_2$	1	Breeds true
		$Pr_1pr_1pr_2pr_2$	2	Segregates for factor Pr_1-pr_1, but phenotypically grey
		$pr_1pr_1Pr_2Pr_2$	1	Breeds true
		$pr_1pr_1Pr_2p_2$	2	Segregates for factor Pr_2-pr_2, phenotypically grey
		$pr_1pr_1pr_2pr_2$	1	Breeds pure

3. SUPPLEMENTARY FACTORS : (Ratio 9 : 3 : 4)—In *Sorghum*, black purple colour of glume is dominant over brown one (P/p). The blackish colour has been found to change into reddish in certain cases. It is explained on the basis of presence of modifying factor Q which has no expression of its own but it reacts with colour producing factor and modifies blackish colour into reddish. The natural colour

of the plant is brown which appears in absence of pigmentation, i.e., when both *P* and *Q* a recessive, the plant colour is expressed as brown. The scheme of the cross may be explained as follows :

Parent Blackish purple PPqq × Brown ppQQ

↓

F_1 Reddish purple PpQq

In F_2, they segregate as follows :

Table 9.3. Supplementary factors

Phenotype	*Frequency*	*Genotype*	*Frequency*	*F_2 behaviour*
Reddish purple	9	*PPQQ*	1	Breeds true (pure)
		PPQq	2	Segregates into 3 reddish purple : 1 blackish purple
		PpQQ	2	Segregates into 3 reddish purple : 1 brown
		PpQq	4	Segregates like F_1
Blackish purple	3	*PPqq*	1	Breeds true (pure)
		Ppqq	2	Segregates into 3 blackish purple : 1 brown
Brown	4	*ppQQ*	1	Breeds true (pure)
		ppQq	2	Segregates for factor *Q-q* but phenotypically brown
		ppqq	1	Breeds true (pure)

The brown glumed parent contains dominant factor *Q* which is ineffective in the absence of *P*. Therefore, two genotypes *ppqq* and *ppQQ* are both brown glumed. The modification of ratio thus arises due to the fact that the last two classes of the former are phenotypically alike and are indistinguishable. Of the two factors involved in this modification, one has visible effect by itself while the other has none by itself which produces a new effect with the interaction of other factor.

4. EPISTASIS : (Ratio 12 : 3 : 1). The phenomenon of epistasis has been noted in cholam (*Sorghum caudatum*). The grain in [illegible]

crop are either pearly or white but in many cultivated types the pearly grains are shining, translucent, lustrous and oily white. Chalky grains are characterised by a large deposit of starch in mesocarp layer. In crosses, pearly is dominant over chalky (*Z-z*) and red colour of the grain is dominant over white colour (*W-w*). When red one is crossed with white pearly, the F_1 plants are red grained. In this case, the pearly or chalky cannot be distinguished due to presence of colour in seed coat, i.e., colour of the grain masks the appearance of grains. It can be shown as follows :

Parents, Red = *WWzz*
White pearly = *wwZZ*
F_1 Red = *WwZz*
Gametes of F_1 *WZ*; *Wz*; *wZ* : *wz*

The results of F_2 can be summarised as follows :

Table 9.4. Epistasis

Phenotype	*Frequency*	*Genotype*	*Frequency*	*F_2 behaviour*
Red	12	*WWZZ*	1	Breeds pure
		WwZZ	2	Segregation into 3 red : 1 white pearly.
		WWZz	2	Segregation of only *Z-z* but phenotypically all progenies red.
		WwZz	4	Segregation like F_1
		WWzz	1	Breeds pure
		Wwzz	2	Segregation into 3 red : 1 white chalky.
White pearly	3	*wwZZ*	1	Breeds pure
		wwZz	2	Segregation into 3 white pearly : 1 white chalky.
White chalky	1	*wwzz*	1	Breeds pure

Thus, epistasis is different from dominance as later relates to allelomorphic pair of factors. In epistasis, the two pairs of factors are independent and are not allelomorphic.

5. INHIBITORY FACTORS (Ratio 13 : 3)—This phenomenon is observed in the inheritance of pigmentation in rice. When a cross is made in between a purple pigmented and a green rice plant, F_1 is green and F_2 shows segregation into 13 green and 3 purple pigmented rice. The

factorial representation of this cross may be

Parents Green *II lp lp*
Purple *ii Lp Lp*
F_1 Green *Ii Lp lp*
Gametes of F_1 *ILp*, *Ilp*, *iLp*, *ilp*

The results of F_2 are summarised here—

Table 9.5. Inhibitory Factors

Pheno type	*Frequency*	*Genotype*	*Frequency*	F_2 *behaviour*
Green	13	*IILpLp*	1	Breeds pure
		IILplp	2	Segregation for *Lp* but phenotypically all green
		IIlplp	1	Breeds pure
		IiLplp	4	Segregation like F_1
		Iilplp	2	Segregation for *Ii* but phenotypically all green
		IiLpLp	2	Segregation into 3 green : 1 purple
		iilplp	1	Breeds pure
Purple	3	*iiLpLp*	1	Breeds pure
		iiLplp	2	Segregation into 3 purple : 1 green

In this cross *Lp* factor produces purple colour in leaves. It is present in one parent. The parent with green leaves possess factor *I* which inhibits the colour production by *Lp*. *I* has no visible effect by itself. So the behaviour of inhibitory factor *I* can be observed by its action on *Lp* as shown in the table.

Examples of inhibitory factors are known in many cases of plants. Similar phenomenon has been observed in the inheritance of coat colour of dog and plume colour in fowls.

6. DUPLICATE FACTORS (Ratio 15 : 1). When two or more factors present on separate chromosomes but execute the same phenotypic action, the phenomenon is called duplicate factors or duplicate genes. The most suitable example is the character pair 'awned-awnless' where in F_2 15 awned and 1 awnless appear.

The factorial representation may be as follows.

Parents Awned $A_1A_1A_2A_2$
Awnless $a_1a_1a_2a_2$
F_1 Awned $A_1a_1A_2a_2$
Gametes of F_1 A_1A_2, A_1a_2, a_1A_2, a_1a_2
The results of F_2 are shown in the table

Table 9.6. Duplicate Factors.

Phenotype	*Frequency*	*Genotype*	*Frequency*	*F_2 behaviour*
Awned	15	$A_1A_1A_2A_2$	1	Breeds pure
		$A_1A_1A_2a_2$	2	Segregation for factors A_2a_2 but phenotypically all breeds pure for awned.
		$A_1a_1A_2A_2$	2	Segregation for factors A_1a_1 but phenotypically all breeds pure for awned.
		$A_1a_1A_2a_2$	4	Segregation like F_1
		$A_1A_1a_2a_2$	1	Breeds pure
		$a_1a_1A_2A_2$	1	Breeds pure
		$a_1a_1A_2a_2$	2	Segregation in 3 awned : 1 awnless
		$A_1a_1a_2a_2$	2	Segregation into 3 awned : 1 awnless
Awnless	1	$a_1a_1a_2a_2$	1	Breeds pure

The table reveals the fact that the two pairs of factors act identically and therefore, first three classes of phenotypes of normal ratio 9 : 3 : 3 : 1 merge into one class ($9+3+3=15$).

Similar case has been observed in chlorophyll inheritance in ragi.

7. POLYMERISM (Ratio 9 : 6 : 1). The phenomenon is observed in case where two dominant factors affect the same trait, i.e., they produce identical effect individually. But when both are present they produce double the effect. It is known as additive effect of the factors. Its suitable example is seen in the inheritance of fruit shape in pumpkin (*Cucurbita melo*). The round shape is dominant and is controlled by factor *A*/*a* or *B*/*b*. When a cross is made between round (*AAbb*) and round (*aaBB*), F_1 is disc shaped because of presence of two dominant factors in one producing double the effect.

Individually *A* or *B* produce round shape but together produce double effect, i.e., fruit is discoid. The factorial representation can be as follows :

Parents Round = *AA bb*
Round = *aa BB*
F_1 Discoid = *Aa Bb*
Gametes of F_1 *AB*; *Ab*; *aB*; *ab*

The F_2 result shows that two middle classes of the normal ratio 9 : 3 : 3 : 1 merge into one and thereby the ratio is modified into 9 : 6 : 1 (polymerism).

8. LETHAL FACTORS : Some factors when present together produce harmful effect and even may cause death to the progeny. For example, if a pair of factors inhibits the chlorophyll synthesis, an autotrophic plant will die due to starvation. The development of chlorophyll is controlled by a single factor. The phenomenon has been observed in snapdragon, maize, rice, cholam etc. where double dominant gene produce lethal effect.

The following cross in Snapdragon will reveal the effect of factors.

Parents Golden Gg
Golden Gg

Hybrids produced from the cross will have genotype

GG	Gg	Gg	gg
Pure golden dies	Golden	Golden	Green

The ratio will be 2 golden yellow : 1 green

The result shows that G in homozygous condition (GG) has a lethal effect. The seedlings in this case will be completely yellow and there will be no development of chlorophyll.

Similar case has been observed in mice coat colour where pure yellow mice dies and ratio therefore comes 2 hybrid yellow : 1 pure non-yellow.

9. PLEOTROPY—When two factors control two or more characters in expression, the phenomenon is termed as pleotropy. It has been established in *Drosophila melanogaster*.

10. MULTIPLE FACTORS OR QUANTITATIVE INHERITANCE OR CUMULATIVE FACTORS

The traits described earlier were qualitative types and the classes could be recognised just by casual looks. Quite many characters do not conform to this pattern as they could not be recognised so easily. Such characters show continuous variation between the two extremes and their characters are not sharply differentiated. Kolreuter for the first time noted this kind of inheritance in tobacco when he managed a cross between a dwarf variety and tall variety and found F_1 intermediate between two parents. There was no dominance. The results of F_2 were also not standard, i.e., a few plants were dwarf and some tall but the majority showed a wide range of variation between two extremes. Thereafter, geneticists and plant breeders obtained many examples that showed a wide range of variation from one extreme to the other. Such traits were generally heights, weight and size of grain, yield, number of seeds, number of grains per ear, length of ears, number of rows of kernels in maize, cold resistance, drought resistance, resistance to lodging etc. Since these characters can be expressed in terms of units of length, weight, time, numbers etc., these are referred as quantitative characters. In animals such examples are milk production in cows and egg laying capacity in chickens. Environment also has greater influence to these characters as compared to qualitative ones.

The inheritance of quantitative characters has been explained by Nilsson-Ehle while working on red kerneled and white kerneled wheat. He assumed that quantitative characters are influenced by a number of independent genes, each gene having small and similar effect while the effect of other genes is additive. That is to say that the effect of one gene is duplicated by the presence of second gene and if there are more genes, the effect is relatively increased. Such genes are described as multiple genes or polygenes.

Nilsson-Ehle (1908) managed a cross in between a red kerneled variety with white kerneled variety of wheat. He obtained intermediate type (medium red) of plants in F_1 while in F_2 the ratio was 15 red : 1 white.

The red plants of F_2 were not uniform as regards to colour. The results of the cross are shown as follows:

Parents Dark red $R_1R_1R_2R_2$ × White $r_1r_1r_2r_2$

$\downarrow$

F_1 generation Medium red $R_1r_1R_2r_2$

F_2 Generation

	R_1R_2	R_1r_2	r_1R_3	r_1r_2
R_1R_2	$R_1R_1R_2R_2$ Dark red	$R_1R_1R_2r_2$ Deep red	$R_1r_1R_2R_2$ Deep red	$R_1r_1R_3r_2$ Medium red
R_1r_2	$R_1R_1R_2r_2$ Deep red	$R_1R_1r_2r_2$ Medium red	$R_1r_1R_2r_2$ Medium red	$R_1r_1r_2r_2$ Pale red
r_1R_3	$R_1r_1R_2R_2$ Deep red	$R_1r_1R_2r_2$ Medium red	$r_1r_1R_2R_2$ Medium red	$r_1r_1R_2r_2$ Pale red
r_1r_2	$R_1r_1R_2r_2$ Medium red	$R_1r_1r_2r_2$ Pale red	$r_1r_1R_2R_2$ Pale red	$r_1r_1r_2r_2$ White

So F_2 generations may be classified into 5 categories—

(i) Dark red—4 dominant genes	1
(ii) Deep red—3 dominant genes	4
(iii) Medium red—2 dominant genes	6
(iv) Pale red—1 dominant gene	4
(v) White—no dominant gene	1
	16 (ratio 1 : 4 : 6 : 4 :1)

Under this category some cases of *transgressive segregants* are also met with in which case parents are not true homozygous and as a result, in some progenies, more intense colour than parents may appear. Based on these studies Nelsson-Ehle proposed a hypothesis which assumes that "a given quantitative character is controlled by a series of independent genes which are cumulative in effect."

Some interesting events of polygenic inheritance are also seen in human race, e. g., skin colour and intelligence. *Skin colour*—Davenport (1910) proposed that human skin colour is controlled by 2 gene pairs. A pure 'Negro' would have the pigment genes $P_1P_1P_2P_2$ and his colour value being very dark while a pure white would have p_1p_1 p_2p_2 with light colour skin. A marriage between these two would give F_1 with $P_1p_1P_2p_2$ intermediate between two parents. These would be mulattos. Intermarriage between mulattos would produce F_2 and the expected result may be as follows :

Parents Black $P_1P_1P_2P_2$ X White $p_1p_1p_2p_2$

↓

F_1 Mulattos $P_1p_1P_2p_2$

Intermarriages between F_1

Mulatto $P_1p_1P_2p_2$ × Mulatto $P_1p_1P_2p_2$

Phenotype	*Genotype*	*Genotype ratio*	*Phenotype ratio*
Black	$P_1P_1P_2P_2$	1	1
Dark	$P_1p_1P_2P_2$	2	
	$P_1P_1P_2p_2$	2	4
Intermediate	$P_1p_1P_2p_2$	4	
	$P_1P_1p_2p_2$	1	
	$p_1p_1P_2P_2$	1	6
Light	$P_1p_1p_2p_2$	2	
	$p_1p_1P_2p_2$	2	4
White	$p_1p_1p_2p_2$	1	1

The number of genes responsible for the skin colour remained a matter of dispute. While Davenport proposed 2 pairs of genes, Gates proposed 3 pairs of genes and Stern opined the presence of 4, 5 or even 6 pairs of genes involved in skin colour inheritance. The matter becomes still more complicated when the environmental effects such as that of sun, wind, cosmetics etc,. are visualised. Besides, in mechanism of inheritance, some genes act as basic colour genes, while others as inhibitor, modifier etc. The inhibitors genes may cause albinism in which the skin, the hair and the eyes become colourless.

Like colour of the skin, height of the man is also an example of polygenic inheritance being inherited by more than 10 pairs of genes. However, tallness is recessive to dwarfness.

Several other examples of polygenic inheritance are now on record such as ear length in maize (Emerson and East, 1916); length of internode in barley spike (Wexelsen, 1933); size of tomato (Lindstrow, 1926); corolla length in *Nicotiana longifolia* (East, 1916); fruit shape in *Cucurbita melo* etc.

GENERAL QUESTIONS

1. Write a critical account of interaction of genes.
2. Write short notes on :—
    (i) Incomplete dominance,
    (ii) Epistasis,
    (iii) Duplicate factors,
    (iv) Multiple factors.

■ ■ ■

CHAPTER 10

CHROMOSOMAL ABERRATIONS

(Architectural changes or Structural changes in the chromosomes)

Each chromosome has got its definite organisation particularly that of genome and a change ,when it breaks, may occur. Such a break may occur due to radiations or chemicals and occasionally under natural conditions. However, the cause of breakage in many cases is undefinable. The recently broken chromosomes behave as if they are sticky and have tendency to adhere to one another. Barbara McClintock (1941) has shown clearly the **Breakage-fusion-bridge** cycle in corn. The segment of chromosome breaks. The broken segment may fuse to the same chromosome in a changed sequence or in other chromosome. The fused segments are unable to separate readily. Such a change may bring out differences between the homologous chromosomes and are detected easily during the cell division. They, thus, form the cytological proof for abberration.

Four types of chromosomal aberrations are now known—Deficiencies, Duplications, Translocations and Inversions.

1. Deficiencies

Bridges discovered the first case of deficiency in 'notched wing' type in *Drosophila*. A deficiency involves the detachment and loss of a portion of an arm from the chromosome. It may be either terminal or interstitial. In terminal deficiencies, there is a single

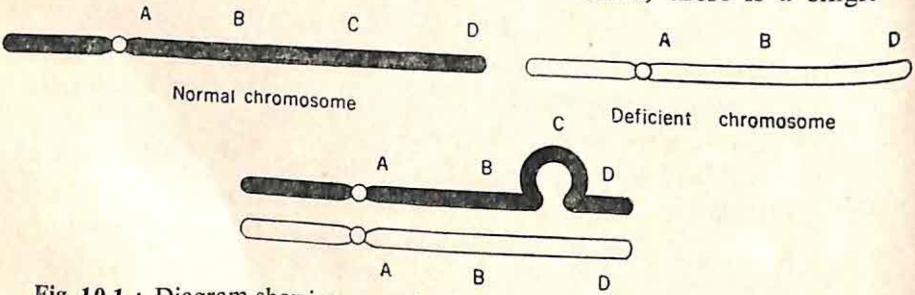


Fig. 10.1 : Diagram showing normal and deficient chromosomes. The lower diagram shows pairing in deficient heterozygote. The region C of normal chromosome bulges out because it has no corresponding part.

break in the chromosome while in the interstitial two breaks followed by reunion occur. The detached portion, if without centromere, will lose the power of movement in anaphase and will be lost. The detached part of chromosome with centromere functions as a deficient chromosome. Deficient chromosome creates difficulty in synopsis and thus brings many significant changes.

Suppose a normal chromosome has a genome *ABCD*, then a deficient one may be *ABC* (terminal deficiency) or *ABD* (interstitial deficiency). In case of interstitial heterozygous deficiency (where one chromosome of the homologous chromosome pair shows deficiency), the deficient chromosome meets the normal one during synopsis. Pairing occurs between strictly homologous segments and as a result an unpaired loop will be formed in the normal chromosome (fig. 10.1). It can be seen in the salivary gland cells of *Drosophila* showing 'somatic pairing' of chromosomes.

Terminal deficiencies have been observed in *Zea mays*.

A deficient chromosome shows missing of a segment as well as genes present in that portion. It thus involves loss of genes which often brings deleterious effects on the individuals. Even minor deficiencies cause detectable morphological abnormalities. Individuals with fairly large number of genes missing are usually non-viable. Deficiencies in homozygotes are mostly lethal.

Classical example of deficiency are found in *Zea mays* and *Drosophila*. In *Z. mays*, the aleurone colour is due to presence of genes *Ccc*. The loss of *C* results in a variegated aleurone with patches of both coloured and colourless tissues. Similarly the mutant character 'yellow body' is produced in *Drosophila* due to minor homozygous deficiency of *X*-chromosome.

Deficiencies are often used in the physical mapping of chromosomes.

2. Duplications

Bridges (1911) discovered duplication in *Drosophila*. He defined that if, in addition to a complete chromosome set, there is an extra piece of chromosome, it is called duplication. This extra piece of a chromosome may be attached to some chromosome or it may exist as an independent fragment. For example, if a genome of a normal chromosome is *ABCDEF* and if two genes *D* and *E* are duplicated, a duplicated chromosome will be *ABCDEDEF* or

ABCDEEDF. A good example of duplication is the position effect on bar eye in *Drosophila* where a gene for eye shape and development is located in *X*-chromosome. A normal eye has 780 facets and is round in shape. The two other types of eyes found are— Bar eye and Bar double eye. A bar eye has nearly 325 to 358 facets and is irregular in shape while bar double eye has 200 facets and is relatively quite reduced in size. Bar eye is the example of duplication in which case genetic locus responsible for the normal eye occurs twice adjacent in the chromosome segment. Similarly bar double eye occurs due to triplication of the same genetic locus. This means that a particular segment is represented once in a normal chromosome, twice in a bar and three times in a double bar. Chromosomes responsible for bar double eyes is observed specially well in salivary gland polytene chromosome of *Drosophila*. These examples also elucidate the position effect of gene and are evidences for a correlation of genetic behaviour with specific cytological events. Besides, Bridges (1935) considered duplication as one cause of evolution which have set in in the nature.

Duplications are comparatively less deleterious than those of deficiencies to the individuals and hence are more frequent. They can, however, be detected due to presence of various abnormalities in morphological characters exhibited by the individuals carrying them.

3. Translocations

Translocation was first discovered by Bridges (1923) in *D. melanogaster*. Srb et al. (1965) defined that *the kind of aberration in which a frequent of one chromosome becomes attached to a non-homologous chromosome is called a translocation*. The simple translocation involves the transfer of the end of one chromosome to

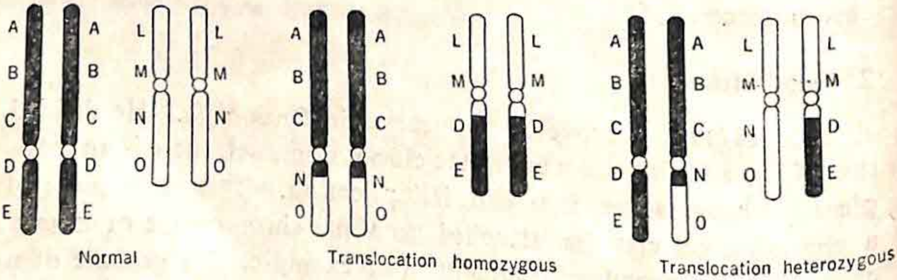


Fig. 10.2 : Diagram showing normal, translocation homozygous and translocation heterozygous chromosome compositions.

the end of non-homologous chromosome. For example, if the genome of the two non-homologous chromosomes is *ABCDE* and *LMNO*, the translocated chromosomes may have *ABCD* and *LMNOE*. It's rare in nature. The other kind of translocation is the reciprocal translocation in which case non-homologous chromosomes may interchange parts, i.e., mutual exchange of the segments between non-homologous chromosomes. If the genomes of normal non-homologous chromosomes are *ABCDE* and *LMNO*, the reciprocal translocation may have genome *ABCNO* and *LMDE*. The reciprocal translocation may exist in either a homozygous or heterozygous state. The chromosomes with homozygous translocation behave normally and do not show any cytological peculiarities. They show a regular pairing at meiosis.

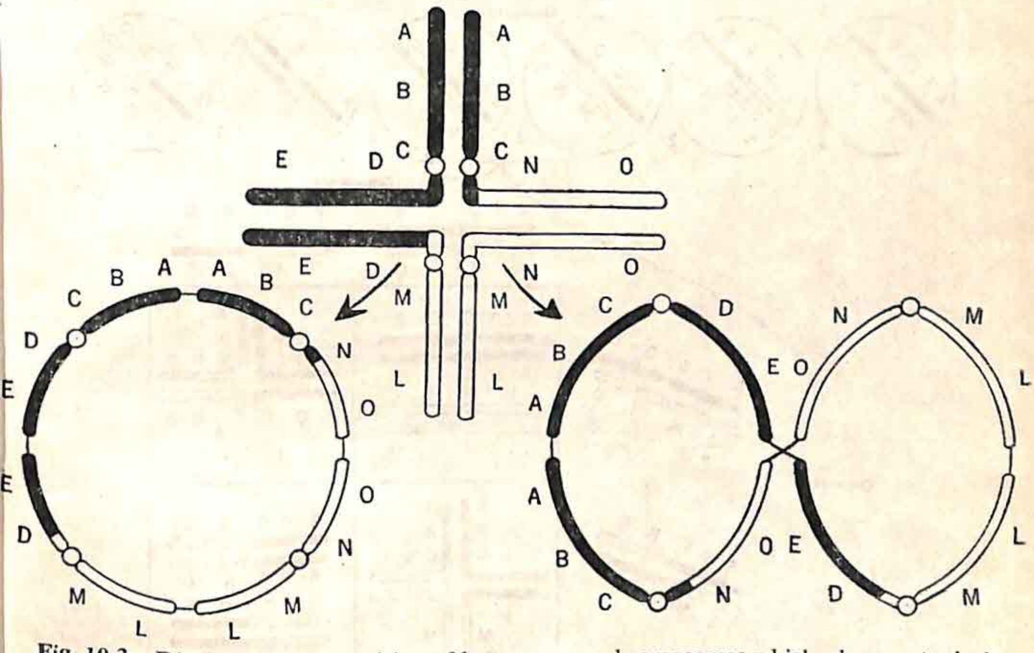


Fig. 10.3 : Diagram showing pairing of heterozygous chromosomes which shows a typical cross-like configuration at pachytene (centre above) and its separation later on. It may open into a ring (lower left) or a figure eight (lower right).

The chromosomes with heterozygote translocation show complicated cytological behaviour and are readily detected by their cross-shaped pairing configurations at pachytene. Later it may open out in a ring or in a figure of eight or zig zag. By the position of cross, the point which had broken to produce translocation can be determined. Heterozygous translocations have been observed in *Zea mays*, *D. melanogaster*, *Oenothera lamarckiana*, *Paeonia*, *Allium sepa*. In *O. lamarckiana*, multiple heterozygous translocations have been found.

The genetic consequences of translocations are also quite significant as shown in fig. 10.4. Some of the gametes formed by heterozygous translocations may carry duplications for some and deficiencies for other. Such gametes either abort or produce inviable zygotes and may bring characteristic semisterility in the individuals. When observed under microscope some of the pollens of semisterile

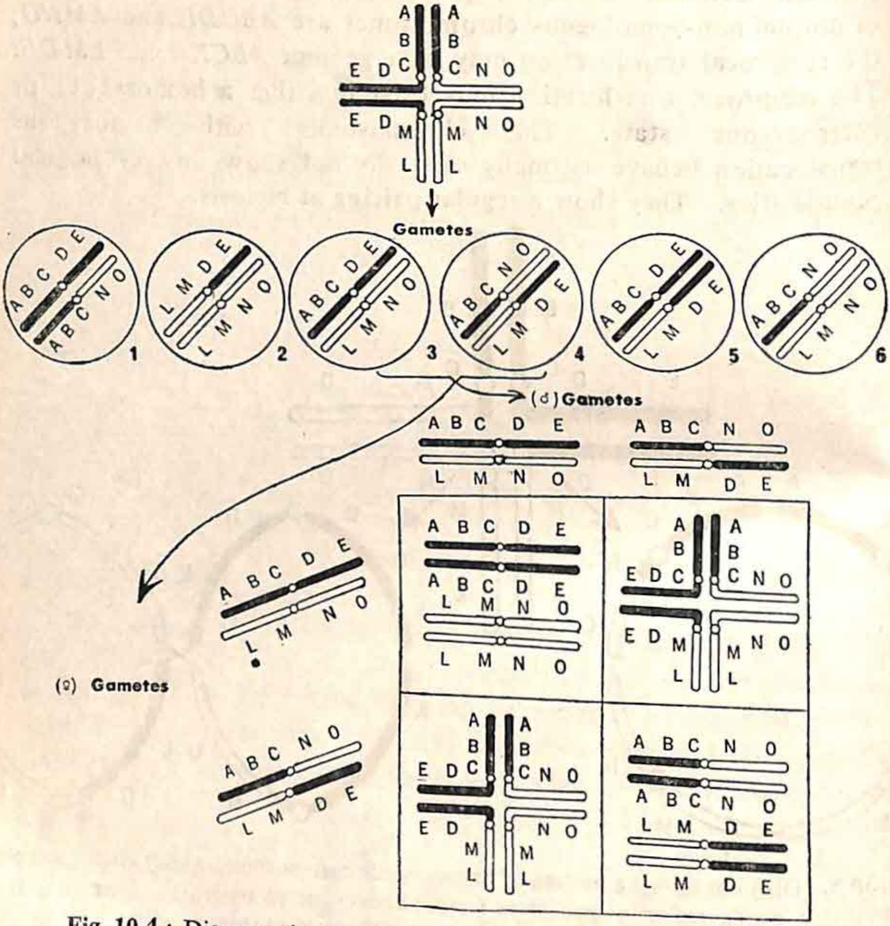


Fig. 10.4 : Diagram showing gamete formation and the result of self-fertilisation of a heterozygous translocation. The offsprings show 1 homozygous standard : 2 translocation heterozygotes : 1 translocation homozygote.

Zea mays are abnormally small and when tested they are found deficient in starch content. This physiological defect occurs due to translocation.

4. Inversions

Inversion is the most complicated type of chromosomal aberration. It was discovered by Sturtevant (1921) in *Drosophila*. It is commonly defined in a way that the term inversion is given to the kind of aberration in which a chromosomal segment exists

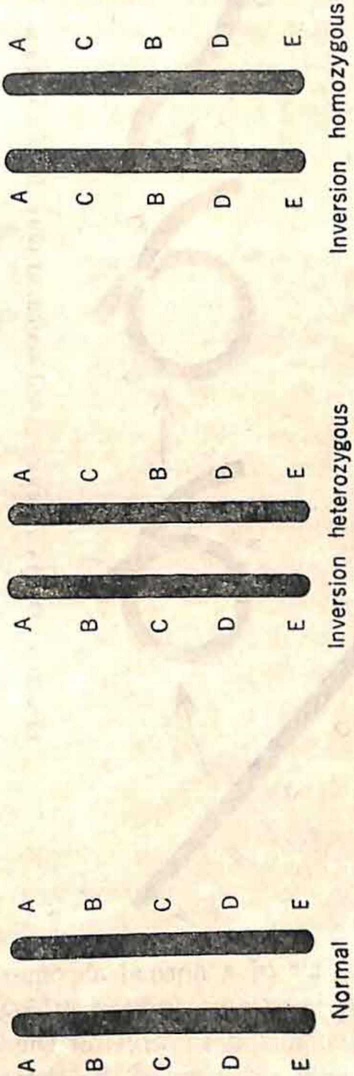


Fig. 10.5 : Diagram showing normal (homozygous standard), inversion heterozygous and inversion homozygous chromosomes.

in reverse relationships to the rest of its chromosomes. In such chromosome, a portion of gene sequence is rearranged in reverse order.

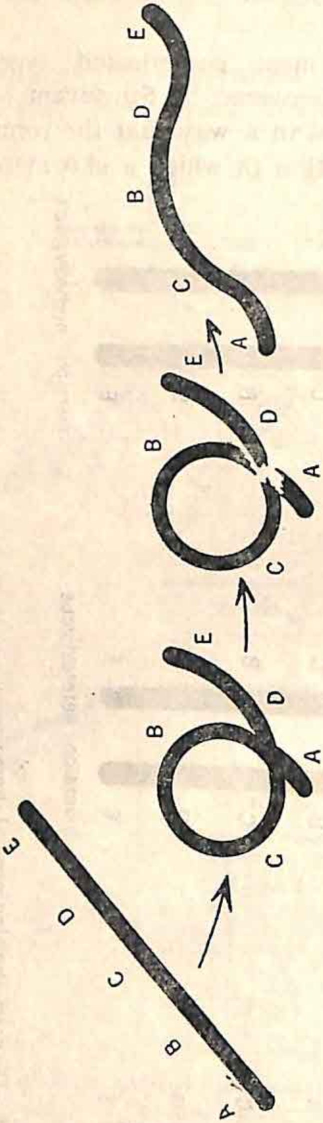


Fig. 10.6 : Diagram showing possible manner of origin of inversions.

For example, if a genome of a normal chromosome is *ABCDE*, the chromosome showing inversion may have *ACBDE*. An inversion may be homozygous (includes both arms of the chromosome) or heterozygous (includes only one arm of the chromosome). If the portion of an inversion includes centromere, it is called pericentric inversion and if centromere is outside the inversion, it is called paracentric. Both these inversions have their own significance in their genetic consequences (fig. 10.7).

In heterozygous inversion, true synapsis is not possible by simple linear pairing. Synapsis occurs by the formation of an inversion loop. In maize and salivary gland chromosome of *Drosophila*, such inversion loops are observed in pachytene stage which are helpful to determine the extent of an inversion. However, if loop formation is not possible, the inverted segment does not succeed in pairing with the portion of a normal chromosome homologous to it.

Crossing over within the inversion loop in such cases results in

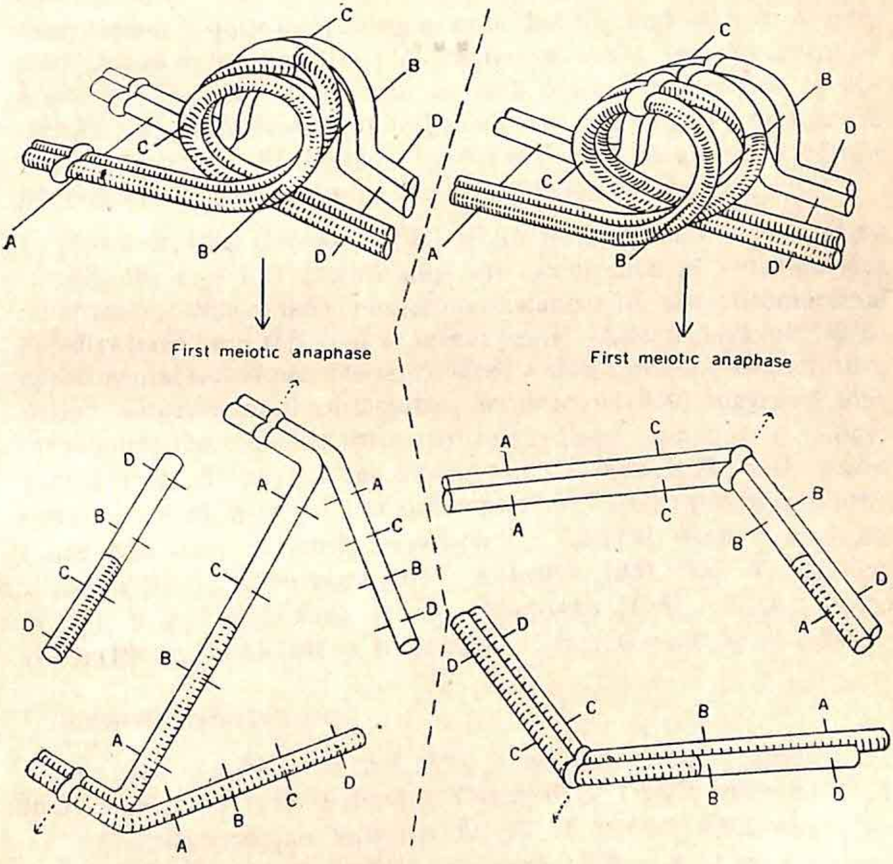


Fig.10.7 : Diagram showing crossing over and the resultant chromosomes after separation. Left half of the diagram shows the condition when the centromere is not involved in inversion (paracentric inversion) and right half of the diag. shows when centromere is involved in the inversion (pericentric inversion).

aberrant chromatids i.e., dicentric chromatids, acentric fragments and chromatids carrying duplications and deficiencies. Gametes with such abnormalities either fail to function in fertilisation or fail to form viable zygotes.

GENERAL QUESTIONS

1. Write an essay on structural changes in the chromosomes.
2. What are the chromosomal aberrations ? Discuss critically the genetic consequences of these changes.
3. How would you distinguish cytologically,
    (i) Paracentric and pericentric inversions
    (ii) Homozygote and heterozygote translocations,
    (iii) Translocations and inversions ?

■ ■ ■

CHAPTER 11

EXTRA-NUCLEAR INHERITANCE

[Cytoplasmic Inheritance]

Mendel proved that both male and female contribute equally to the heredity of an offspring. This idea later led to develop **the gene theory** and ultimately from the very inception of science of genetics, chromosomes were considered the sole agent of heredity. At the same time it is quite interesting to note that the importance of cytoplasm for carrying hereditary characters was not ignored even by Mendel or Darwin. Cytoplasm as such passes from parent to offsprings (i.e., from generation to generation) and acts as a medium to support nucleus. At the same time it may also be a source of raw materials to be needed during further duplication of genes.

However, Carl Correns (1909) while working on *Mirabilis jalapa* earned the credit of establishing the occurrence of extra-nuclear inheritance. Since then numerous instances of non-chromosomal heredity have been reported in recent years. Due to lack of better experimental techniques the work faced a slow progress during that period. Considering the difficulties, Sonneborn (1960) suggested that "it requires the combine forces of embryology, genetics, cytology, microbiology, biophysics and biochemistry to attack the basic problems connected with cellular differentiation." Cytoplasmic inheritance has been variously reviewed by handful workers such as Rhoades (1946), Sonneborn (1951), Ephrussi (1951, '53, '58), Nanney (1957), Briggs and King (1959), Michaelis (1962), Bhan (1964), Beale (1964), Links (1965), Ruth Sagar (1965, '67) and Serra (1968).

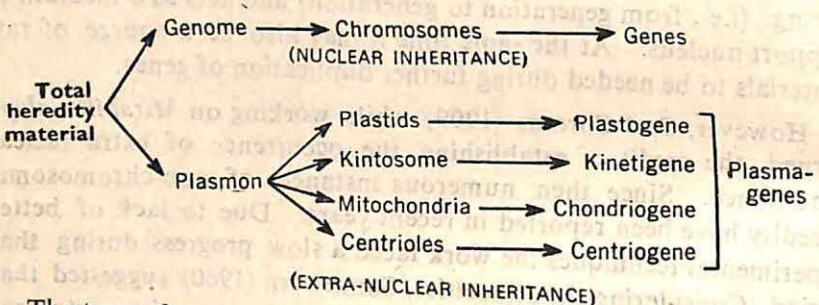
Cytoplasmic genetic system

Earlier it was believed that *DNA* is the sole agent of inheritance in an organism but from the time Colter et al (1965) reported that TMV (Tobacco mosaic virus) is devoid of *DNA*, *RNA* was also included as the second heredity material. Likewise, Carl Correns' contribution on the role of cytoplasm in heredity put the concept of the heredity to an inevitable position of revision. So with the very inception of cytoplasmic, inheritence two types of heredity have to be recognised.

(1) THE NUCLEAR HEREDITY : It includes all sorts of heredity in which nucleus participates and genetic set consists of nucleic acid or nucleoprotein. Here the genetic set of an organism is known as genome.

(2) THE CYTOPLASMIC HEREDITY : It refers to the transmission of heredity by the cytoplasm. The genetic set which collectively consists of cytoplasmic inclusions is termed as plasmon. The function of plasmon is similar to that of genome but former maintains its separate identity from genome because of its failure to follow the Mendelian pattern of inheritance. Plastids, mitochondria, microsomes etc. are self propagating bodies present in the cytoplasm and these play significant roles in cytoplasmic inheritance.

Link (1965) in his monographic contribution divided the total heredity material of a cell as follows.



The term **plasmon** was introduced by Fritz Von Wettstein (1924) to refer the total extra-chromosomal heredity complement of a cell. It is equivalent to genome (chromosomal heredity). The term **plasmagene** was introduced by C. D. Darlington (1939) to represent the smallest heritable unit in the plasmon. The relationship between a plasmagene and the plasmon is thus homologous to that of a gene and genome. Imai (1937) introduced another term **plastogene**. To represent the identical sense certain terms such as kinetogenes, centriogene, chondriogene etc. have also been introduced.

Role of genes in cytoplasmic inheritance

Sonneborn (1951) has recognised five categories of genic behaviour in relation to cytoplasmic inheritance.

(1) INDUCTION OF MUTATIONS IN CYTOPLASMIC GENETIC MATERIAL e.g., iojap inheritance in corn (Rhoades, 1943, '46).

(2) CONTROL OF INTRACELLULAR CONCENTRATION OF CYTOPLASMIC GENETIC MATERIAL e.g., inheritance of kappa particles in *Paramecium*. Genes are known to control the concentration of kappa particles in killer *Paramecium*.

(3) SELECTIVE ACTION ON CYTOPLASMIC GENETIC MATERIAL, e.g., plastid development in *Oenothera*. Here gene acts on alternative cytoplasmic material.

(4) INTERACTION ON CYTOPLASMIC GENETIC MATERIAL, e.g., petite colonies in yeast. Characters of an offspring are obtained as a result of interaction of genome and plasmon.

(5) CONTROL OF SPECTRUM OF ALTERNATIVE CYTOPLASMIC STATES, e.g., homozygous *Paramecium* can produce eight descendents different in the kind of antigen carried on their cilia. The different gene of the same nucleus may produce different phenotypes under certain conditions.

(The details of the example are discussed separately.)

PLASTID INHERITANCE

Imai (1937) has proposed that plastids are autonomous. They possess genetic mechanism of their own and are beyond the control of gene. Chlorophyll variegation in *Croton* is due to the presence of two distinct types of plastids in the cytoplasm of zygote and these undergo a regular segregation. Darlington and Mather (1949) supported Imai's view and suggested that plastogenes are present in plastids which are capable of mutation and giving rise to new chlorophyll characters. The findings were confirmed on *Euglena* by them. In the experiment, when Euglenae were allowed to grow after inducing accidental fragmentation, all plastid containing fragmented parts grew successfully and developed into new and normal plants, but the parts which had no fragments of plastids failed to grow and died.

Weir and Stocking (1952) recognised four categories of plastids considering their inheritance behaviour.

(A) Plastids may change characters by gene-controlled mutation and then the changed plastids are inherited independent of nuclear control. This category can be explained while elucidating the example of "plastid mutation in corn." Rhoades reported that this change is caused by recessive iojap gene.

ZEA MAYS : Rhoades in his studies has tried to establish the relation between a plastid abnormality and the regularly transmitted genes. Although many kinds of plastid abnormalities have been observed, but easily determinable ones are in which the plastids are sectored, i.e., containing normal gene as well as mutated stripes. Both chromosomal and non-chromosomal factors are known to induce stripes. The non-chromosomally induced striping is called "*status albomaculatus.*" Rhoades made reciprocal crosses between normal green and striped plants and observed that in crosses with female parent normal green, the striping phenotype disappears and all progenies are green and chromosomal gene segregate normally

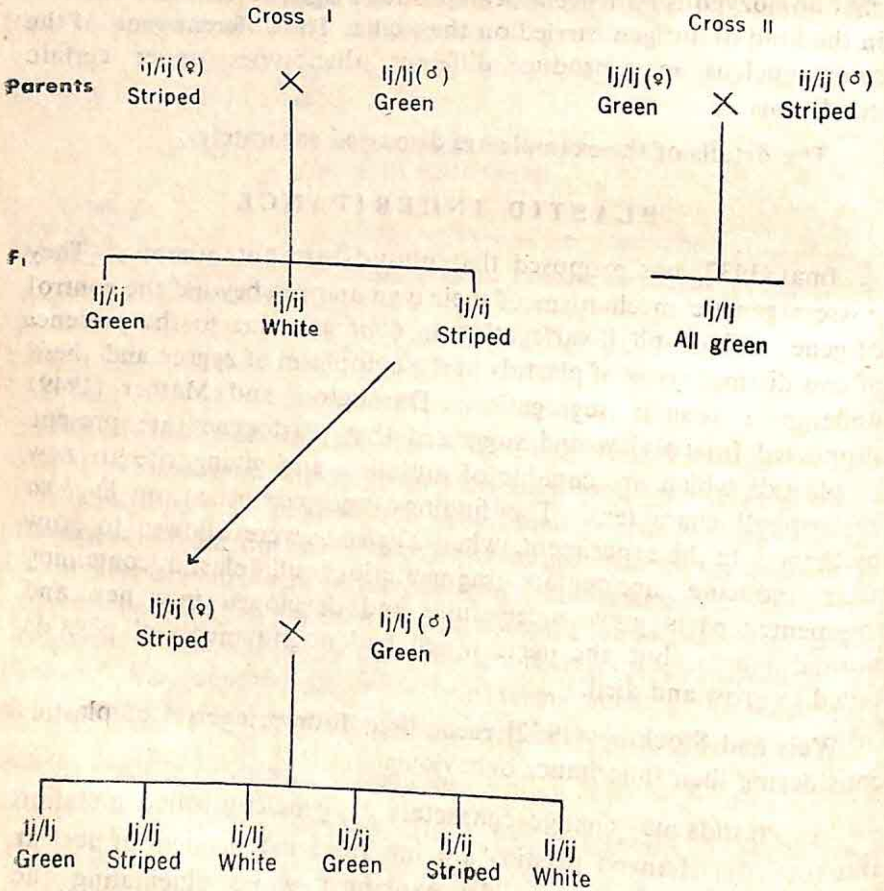


Fig. 11.1 : Results of reciprocal crosses between striped (iojap) and green *Zea mays* plants and back cross of F_1 striped by normal green plants.

in F_2 generation. When female parent is striped, there appears three types of F_1 progenies—colourless, striped and green with variable frequencies. When F_1 striped plants are self pollinated or crossed to any male, three types of progenies appear again and chromosomal genes segregate normally.

A detailed study of iojap mutants of maize has been made by Rhoades. Iojap is a chromosomal gene. It gives rise to striped plants when homozygous recessive. Such striped plants when crossed reciprocally with green ones, the progeny seedlings exhibit maternal inheritance of striping (fig. 11.1). It has been recorded that in many generations of back crossing striped females with green males, there is no effect of the male parent upon the progeny. Such characteristic feature is followed even in the absence of iojap gene after outcrossing. Rhoades observed that the gene iojap produces mutation, i.e., an irreversible change in a non-chromosomal determinant. It is further supported from the point that one does not find cells with mixed green and mutant plastids at the boundaries of striped areas.

The iojap gene is not unique. Similar abnormal plastid phenotypes are also known in *Nepeta*, barley and rice.

(B) As independent units plastids may interact with nucleus and other protoplasmic constituents to determine the chloroplast characters. Common example is the plastid development in *Oenothera*.

OENOTHERA : Renner (1936) demonstrated the interaction between the plastids and nucleus in *Oenothera*. He observed the two sets of chromosomal complexes one in *O. odorata* (*V* & *I*) and other in *O. berteriana* (*B* & *i*). Wagner and Mitchell (1955) advanced his studies by making reciprocal crosses of these forms and observed significant results as given in the table.

Table 11.I. Cytoplasmic inheritance in *Oenothera.*

	Progeny from crosses	
Chromosomal complex	*O. berteriana* × *O. odorata*	*O. odorata* × *O. berteriana*
	♀ *Bi* ♂ *VI*	♀ *VI* ♂ *Bi*
BV	Non-viable	Viable
BI	Normal	Weak and yellow
iV	Mostly normal, Lower leaves some times yellow	Weak and yellow
iI	,, ,,	Non-viable.

The result shows the differences in qualitative expression of plastids. The table shows that *odorata* plastids do not develop properly with chromosomal complex *BI* and *iV* and show interaction as independent units. Thus, there is a marked difference in the qualitative expression of plastids.

(C) Plastids behave as independent and autonomous units. These are capable of duplicating themselves similar to choromosomes. Common example is the leaf variegation in *Mirabilis jalapa* (4 O'clock plant).

Mirabilis Jalapa : It serves as the most common example of non-Mendelian heredity. Correns (1909) while working on the problem evidently substantiated the role of cytoplasm in heredity. It was the first example of cytoplasmic inheritance which challenged the Mendelian heredity. Correns described it as *albomaculatus type of variegation*. The results of his detailed experimental findings are summarised in the table 11.II and figure 11.2.

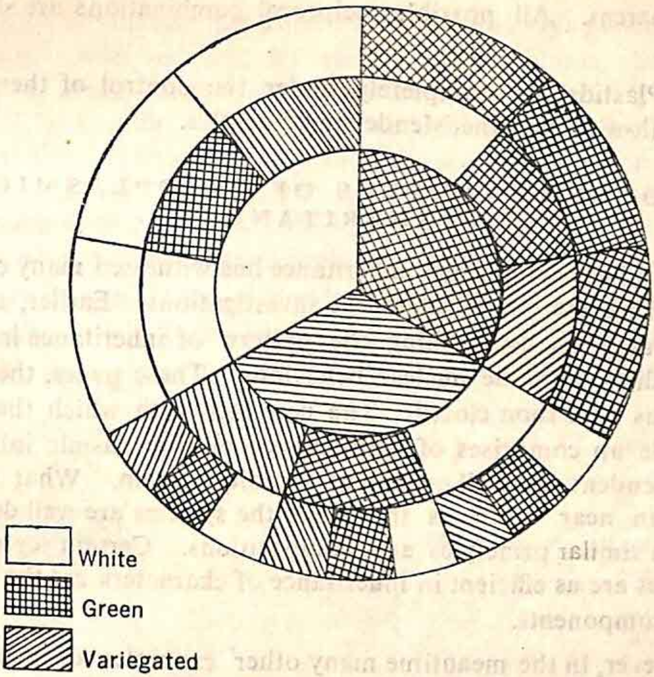


Fig. 11.2 : Diagrammatic representation of cytoplasmic inheritance in *Mirabilis jalapa.* Centre circle represents male branch, middle circle to female branch and outer to progenics.

Table 11. II. Inheritance of plastid characters in *M. jalapa.*

Pollen from the branch or Type ♂	*Pollinated flowers on branch or Type ♀*	*Progeny grown from seed*
Pale	Pale Green Variegated	Pale Green Pale, green and variegated
Green	Pale Green Variegated	Pale Green Pale, green and variegated
Variegated	Pale Green Variegated	Pale Green Pale, green and variegated.

A perusal of the table makes it evident that the plastid inheritance in the progenies solely and invariably depends upon the type of female parent. All possible reciprocal combinations are shown in the table.

(D) Plastids are completely under the control of the nucleus. These follow strictly the Mendelian principles.

OTHER EXAMPLES OF CYTOPLASMIC INHERITANCE

The subject cytoplasmic inheritance has witnessed many changing faces during the course of recent investigations. Earlier, scientists concentrated on establishing the pattern of inheritance in view of relationships with the nuclear behaviour. These pages, these interpretations were soon closed. The new face with which the subject has come up comprises of maintenance of cytoplasmic inheritance as independent and well organised heredity system. What seems to appear in near future is that both the systems are well developed based on similar principles and compositions. Certain cytoplasmic organelles are as efficient in inheritance of characters as the nucleus and its components.

However, in the meantime many other examples of cytoplásmic inheritance have sprang up and a few of them are discussed as follows :

1. Influence of Cytoplasm on genome in *Epilobium*

Michaelis and his co-workers (1951, '59, '62) have studied extensively the inheritance of morphological characters in interspecies crosses of genus *Epilobium*. In a reciprocal cross between *E. hirsutum* (race 4) and *E. parviflorum*, he observed that when *E. hirsutum* is taken as female, the offsprings are always sterile and stunted with crippled leaves while offsprings grow 4 feet tall, possess normal leaves and flower profusely when *E. parviflorum* is taken as female. The results indicate that hybrid nucleus seems to be incompatible with cytoplasm of *E. hirsutum*. Michaelis (1962) has therefore suggested to consider the entire set up of the cell in cytoplasmic inheritance.

2. Killer Characters in *Paramecium*

Sonneborn (1949) observed that one strain of *Paramecium aurelia* kills the other strain of the same species by secreting a toxic substance called paramacin. He called the former strain, the **killer** and the later one the **sensitive**.

During reproduction, the killer *Paramecium* always produces killer individuals while sensitive to sensitive individuals. Sonneborn managed a cross between killer and sensitive strains. The results were quite interesting. If the mating between two individuals is short enough as to permit the exchange of nucleus only, the killer produces killers and sensitive only sensitives. However, if conjugation lasts long enough as to permit the mixing of the cytoplasm along with the exchange of nuclei both the exconjugants are killers. These crosses, thus make it clear that property of being killer depends upon cytoplasmic particles called **kappa**, and the nuclear genes which are exchanged during mating have no effect. Sonneborn has further

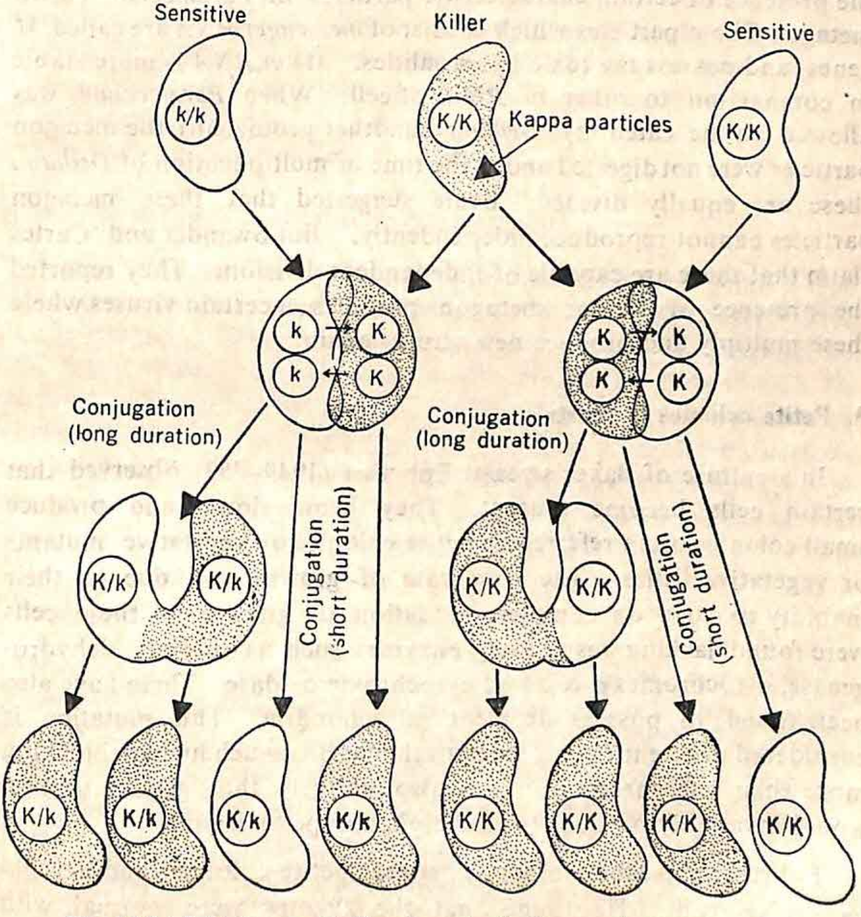

Sensitive
Killer
Sensitive
k/k
K/K
Kappa particles
K/K
k
k
K
K
K
K
K
K
Conjugation (long duration)
Conjugation (long duration)
Conjugation (short duration)
Conjugation (short duration)
K/k
K/k
K/K
K/K
K/k
K/k
K/k
K/k
K/K
K/K
K/K
K/K

Fig. 11.3 : Inheritance of killer character in *Paramecium aurelia*. An animal must contain kappa particles and the dominant gene K to become a killer.

shown that the effect of kappa particles reduces considerably in the absence of gene *K* (dominant) and gradually killing potentiality disappears. It means that kappa particles cannot be produced by the nucleus alone or by the cytoplasm alone.

So in killer, the ability of killing is due to certain particles called **kappa** found in the cytoplasm. These particles pass on from generation to generation in these strains. Such particles are not found in the cytoplasm of the sensitive strain. The recent investigation has shown that kappa particles contain *DNA*, *RNA* and protein. Its maintenance and production of paramecin depend upon the presence of dominant nuclear gene *K*. Besides, Beale (1964) reported the presence of certain characteristic particles in *Paramecium* called **metagon.** These particles which consist of *messenger RNA* are called *M* genes and possess the toxic potentialities. Its *m RNA* is more stable in comparison to other *m RNA* of cell. When *Paramecium* was allowed to be eaten by *Dedium* (another protozoan), the metagon particles were not digested and at the time of multiplication of *Dedium*, these are equally divided. Beale suggested that these metagon particles cannot reproduce independently. But Swander and Cartes claim that these are capable of independent division. They reported the presence of similar metagon particles in certain viruses where these multiply and produce new viruses again.

3. Petite colonies in yeasts

In a culture of Baker's yeast, Ephrussi (1949, '53) observed that certain cells become mutant. They grow slowly and produce small colonies often referred as petite colonies or vegetative mutants or vegetative littles. The slow rate of growth was due to their inability to carry on complete oxidation of glucose as these cells were found lacking respiratory enzymes such as succinic dehydrogenase, cytochromes *a* & *b* and cytochrome oxidase. These have also been found to possess deficient mitochondria. This mutation is considered different from gene mutation as it is much higher (blocking more than one enzymes). It is also unlikely that gene mutation would invariably produce the same phenotype each time.

Ephrussi managed a cross in between petite colonies and normal vegetative cells. He found that the zygotes were normal with respect to respiration. In F_2 generation, all the progenies were normal which would mean that the mutant characters of vegetative littles were lost when these cells fuse with normal gene cells. Had it been a deficiency of a gene mutation, it should have reappeared. He suggested that as soon as vegetative littles receive normal cytoplasm, it becomes reactive and restores normalcy. This again endorses the view that both the normal and vegetative littles possess some genome and the production of petite colonies is an example of cytoplasmic inheritance.

4. Poky characters in *Neurospora*

While working on *Neurospora crassa*, Mitchell and Mitchell (1962) brought out an outstanding evidence in support of cytoplasmic inheritance, when they made a cross between a wild type and other strain and observed the appearance of certain slow growing plants called **poky strains**. Then they made a cross in between slow growing poky strains and wild type and found that poky character is transmitted to all progenies when it is carried by the maternal parent and to none when carried by male parent.

5. Inheritance of CO_2 sensitivity in *Drosophila*

L' Heritier and Teissier in their experiment observed that a true breeding strain of *Drosophila melanogaster* is carbon dioxide sensitive and develops paralysis or uncoordinated movements when exposed to CO_2 for a short period. They made reciprocal crosses of CO_2 sensitive and normal strain of *Drosophila* and observed that sensitive female flies always produce CO_2 sensitive flies. It has been therefore concluded that the trait of CO_2 sensitive is inherited through the maternal line, i.e., through the cytoplasm. Virus-like particles called **sigma** are considered as responsible for inheriting this trait.

6. Streptomycin resistance in *Chlamydomonas*

Ruth Sager (1965) worked on unicellular green alga, *Chlamydomonas reinhardi* and isolated its two strains, one resistant to 500µg. of streptomycin per ml. and the other wild typé sensitive. She managed reciprocal crossing between two and recorded that the resistance trait in this alga is inherited by female parent, e.g., if mother is streptomycin resistant, all progenies will be resistant. She advanced her studies and noted that mutant types of zygotes are produced in both types of crossings. This led her to believe that extra chromosomal entity (or streptomycin resistant factor) must be present in both the types of parents, but, for some unknown reason, only the contribution of female parent is incorporated into the extra-chromosomal material of progeny. Obviously, there is no physical basis for this uniparental transfer.

Another fascinating point which comes from her studies is the behaviour of extra-chromosomal gene. These genes divide in orientally mechanical type which means, through it, each non-chromosomal gene is transmitted to the offsprings regularly. She noted that ac_1 & ac_2 and *Sd* & *Sr* are extra-chromosomal genes. ac_1 and ac_2 are mutant that block photosynthesis and require acetate in the medium whereas *Sd* and *Sr* are streptomycin dependent and streptomycin resistant respectively.

In her studies, she noted that,

(i) segregation of extra-chromosomal genes begins during the first few doubling after meiosis,

(ii) ac_1/ac_2 pair segregates in an average ratio of 1 : 1,

(iii) ac_1/ac_2 and *Sd*/*Sr* pairs segregate independently of each other in both time and space. (It means that two pairs of extra-chromosomal genes behave as if they are carried on separate particles).

(iv) each daughter cell always receives one or other form of extra-chromosomal genes. In this sense, ac_1 and ac_2 are alleles and so *Sd* and *Sr*.

Experiments on back crossing revealed that new types are genetically stable and, therefore, can be considered as true intragenic cytoplasmic recombinants analogous to the intragenic chromosomal recombinants.

Thus, studies on extra-chromosomal inheritance in *Chlamydomonas* has added a new adiantum to the subject and has invited scientists to explore more facts in this regard.

7. Cytoplasmic male sterility (cytosterility)

Cytoplasmic male sterility or cytoplasmic control of male sterility is known in several crops including *Zea mays*. Its very simple experiment to work out. In such cases if female parent is male sterile, i.e. female inflorescence is fertile and male inflorescence (tassel) is sterile (plant is monoecious), the progeny F_1 will always be male sterile as is shown in the fig. 11.4. It happens because of the cytoplasm which is mainly derived from the egg obtained from the male sterile female parent.

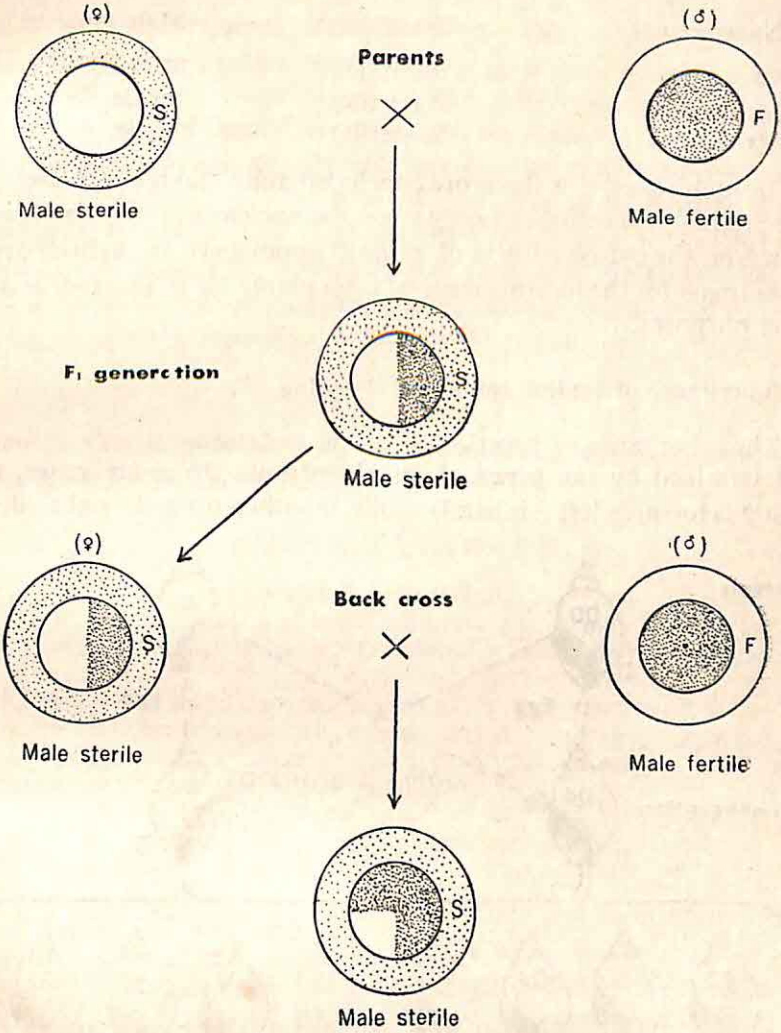


Fig. 11.4 : Diagram showing maternal inheritance of cytoplasmic male sterility. The fact is further substantiated by making back cross. (*S*=sterile male and *F*=fertile male)

While studying the ultrastructure of male sterile cytoplasm, the particles in the size range of viruses have been observed. Whatever these are, these seem to be present only when nuclear genotype is *ms ms* and are lethal in a monoploid cell with the nuclear gene *ms*.

Some crosses with various genomic combinations were made and the following results were found.

Nuclear gene	×	**Cytoplasm**	→	**Male progeny**
ms ms		*S* (sterile)		male sterile
Ms ms		*F* (fertile)		fertile
Ms ms		*S* (sterile)		fertile.

In such cases, it is therefore concluded that though cytosterility is maternally inherited but only when the nuclear genotype is proper. However, the cytosterility is of utmost importance in hybridisation programme for the improvement of crop plants for grain, fodder and other purposes.

8. Inheritance of dextral and sinistral coiling

The inheritance of direction of coiling in *Lemnaea peregra* (snails) is determined by the genes of female parent. In some cases, the coiling is towards left (sinistral) while in other towards right (dext-

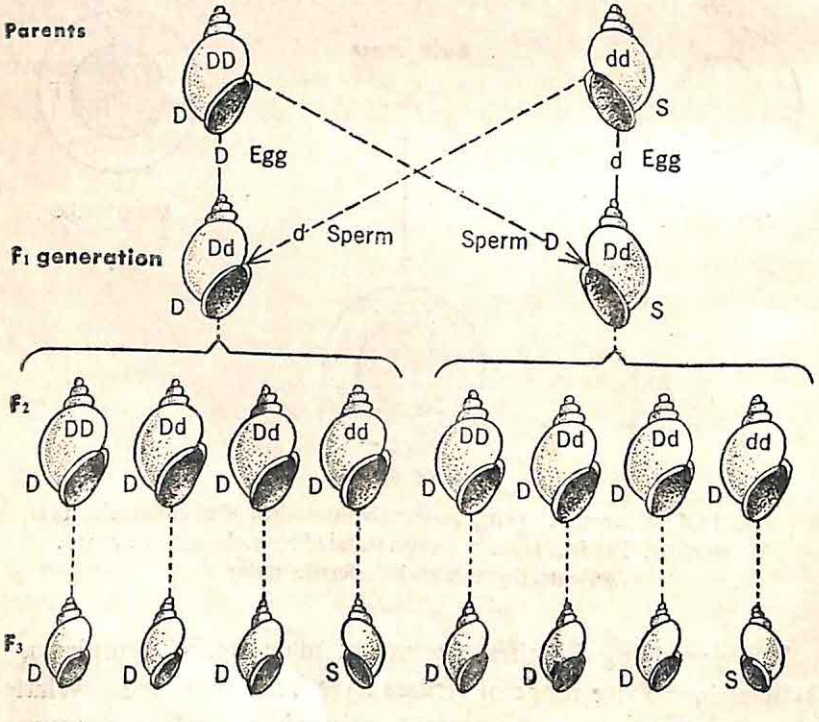


Fig. 11.5 : Inheritance of dextral (*D*) and sinistral (*S*) types of coiling in *Lemnaea peregra.*

ral). In *L. peregra*, the dextral coiling depends upon the dominant allele *D* and the sinistral coiling on recessive allele *d*, so that the dextral is *DD* and the sinistral is *dd*. If dextral type is symbolised by the gene *D* and sinistral type by *d*, a female having *DD* or *Dd* will produce only dextral type of coiling in the offsprings. When a *Dd* female is crossed with *dd* male, 50% offsprings produced would be *dd*. In fact, *dd* should be sinistral but it is not so, but all of them produced dextral coiling. It is so because the *D* gene present in the mother influences the cytoplasm of the egg so as to develop dextral coiling in the offsprings. The *dd* female offspring in its turn will produce only sinistral offspring, as its cytoplasm present in the egg will grow under the influence of *d* gene. This example is also considered as a case of the delayed effect of the genotype.

9. Mouse "milkfactor"

It has been observed that a virus causing mammary cancer in mice is transmitted through the mother's milk.

GENERAL QUESTIONS

1. Write an essay on cytoplasmic inheritance.
2. Discuss the role of gene in relation to cytoplasmic inheritance.
3. What is plastid inheritance ? Describe it critically with the help of suitable examples.
4. Write an essay on cytoplasmic sterility.
5. Write short notes on—(i) Cytosterility, (ii) Inheritance of dextral and sinistral coiling, (iii) Streptomycin resistance in *Chlamydomonas*, (iv) Petite colonies in yeast and (v) Poky characters in *Neurospora*.

■ ■ ■

CHAPTER 12

POLYPLOIDY

Commonly alternation of generation refers to the presence of gametophytic and sporophytic generation one after the other in the life cycle of an organism. The gametophytic generation, which represents the haploid phase of the life cycle, is followed by sporophytic generation or the diploid phase. Haploid phase is symbolised by *n*-set of chromosomes and diploid by 2*n*. In haploid set, there is a single set of homologous chromosomes as in gametes. Gametes fuse to form zygote which behest a diploid set of homologous chromosomes. In the life cycle, the diploid set of chromosomes is reduced to half by the occurrence of meiosis at one stage or other, particularly during the formation of sexual spores. This type of life cycle and the course of chromosomal set are found among typical organisms.

There are several exceptions to this rule and in quite many cases variations in the number of chromosomes are met with. Such examples are quite frequent among mosses, ferns and flowering plants. They are less common among gymnosperms (only found in *Pseudolarix*, *Sequoia*, *Juniperus*) and animals. About one half of the cultivated plants are polyploids. A higher proportion of polyploids is seen among families—Polygonaceae, Crassulaceae, Rosaceae. Malvaceae, Graminae and Iridaceae.

Classification and Terminology

Based on the presence of chromosomal set per nucleus, Serra (1968) recognised two major categories of the organisms.

(1) ORTHOPLOIDS : Organisms showing typical alternation of haploid and diploid chromosomal set in their life cycles have been kept under orthoploids. In such organisms, life cycle dwells between *n* to 2*n* and vice versa.

(2) ANORTHOPLOIDS : Organisms those possess chromosomal sets outside the normal one are called anorthoploids. To this, the word polyploidy is also referred.

Anorthoploids have been further divided as follows :

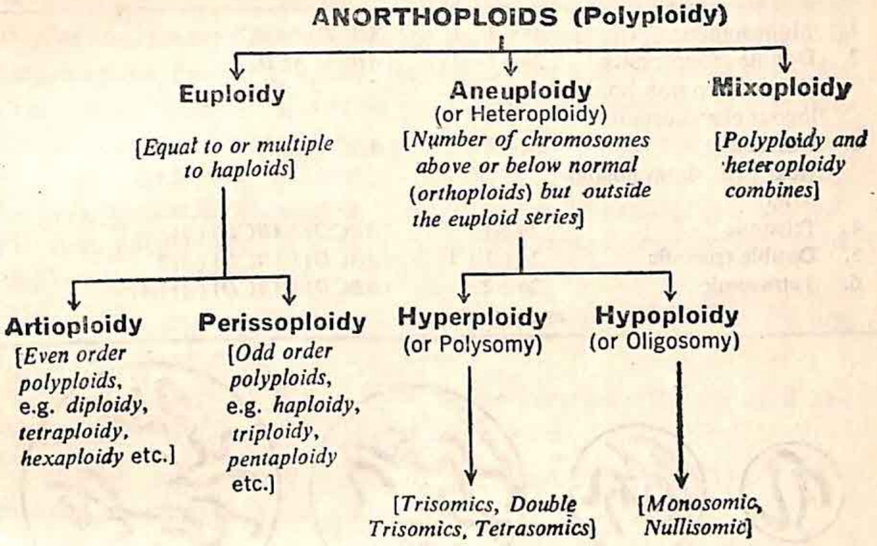

ANORTHOPLOIDS (Polyploidy)
Euploidy
[Equal to or multiple to haploids]
Aneuploidy (or Heteroploidy)
[Number of chromosomes above or below normal (orthoploids) but outside the euploid series]
Mixoploidy
[Polyploidy and heteroploidy combines]
Artioploidy
[Even order polyploids, e.g. diploidy, tetraploidy, hexaploidy etc.]
Perissoploidy
[Odd order polyploids, e.g. haploidy, triploidy, pentaploidy etc.]
Hyperploidy (or Polysomy)
Hypoploidy (or Oligosomy)
[Trisomics, Double Trisomics, Tetrasomics]
[Monosomic, Nullisomic]

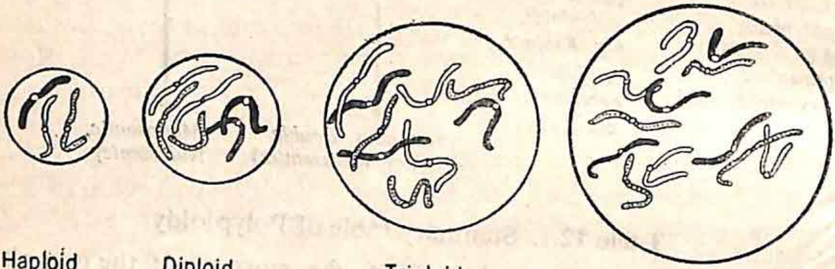
Table 12.I. Summary table of Polyploidy

ABCD = standard haploid set of chromosome of the organism (assumed).

POLYPLOIDY

No.	Type	Chromosomal set	Chromosomal complement of an organism euploidy
1.	Monoploid or haploid	n	$(ABCD)$
2.	Diploid	$2n$	$(ABCD)\ (ABCD)$
3.	Triploid	$3n$	$(ABCD)\ (ABCD)\ (ABCD)$
4.	Tetraploid	$4n$	$(ABCD)\ (ABCD)\ (ABCD)\ (ABCD)$
5.	Autoploidy, e.g., Autotetra ploid (Polyploidy arising from same species)	$4n$	$(ABCD)\ (ABCD)\ (ABCD)\ (ABCD)$
6.	Alloploidy, e.g., Allotetraploid (Polyploidy arising from two different species)	$4n$	$(ABCD)(ABCD)(A'B'C'D')(A'B'C'D')$

Aneuploidy (or Heteroploidy)		
1. Monosomics	$2n-1$	*(ABCD) (ABC)*
2. Double monosomics (loss of two non-homologous chromosomes)	$2n-1-1$	*(ABC) (ABD)*
3. Nullisomic (loss of homologous pair)	$2n-2$	*(ABC) (ABC)*
4. Trisomic	$2n+1$	*(ABCD) (ABCD) (A)*
5. Double trisomic	$2n+1+1$	*(ABCD) (ABCD) (AB)*
6. Tetrasomic	$2n+2$	*(ABCD) (ABCD) (A) (A)*
	and so on	



Haploid Diploid Triploid Tetraploid

Fig. 12.1 : Diagram to represent chromosomal composition of some polyploid forms.

Euploidy

Euploids include all those organisms which have multiplication of whole set of chromosomes, e.g., triploids, tetraploids, pentaploids etc. An ovarian tumour of insect *Drosophila* is 32 ploid. Salivary gland cells of *Gerris* may be 2,000 ploid. Salivary gland cells of many other insects have chromosomes which replicate but do not separate and thus, give rise to polyteny or polytene chromosomes. The beetle *Chironomus* is estimated to have as many as 16,000 chromonemata in a chromosome of metabolically active cells. It is considered as one of the extreme examples of polyteny among the living organisms. Euploidy is very common among plants and may be constituting one-half of the cultivated plants as euploids. Some categories of euploids are discussed here.

Haploids

Haploids are also called monoploids. They have haploid set of homologous chromosomes symbolised as '*n*'. Haploid plants are frequently seen among algae, fungi and bryophytes but are rare in flowering plants. Blakeslee reported the first case of monoploid in *Datura stramonium* in 1922. Since then several cases of haploids in nature among flowering plants have been reported. Some of them are *Brassica campestris*, *B. napella*, *Datura stramonium*, *Gossypium barbadense*, *G. davidsonii*, *G. hirsutum*, *Hordeum vulgare*, *Lycopersicum esculentum*, *Nicotiana tobaccum*, *N. sylvestris*, *Oenothera hookeri*, *O. lamarckiana-gigas*, *Oryza sativa*, *Portulaca grandiflora*, *Triticum compactum*, *T. dicoccum*, *T. persicum*, *T. vulgare*, *Zea mays* etc.

Haploids may be spontaneous or induced and may arise by one of the following ways.

(i) By female parthenogenesis as in *Solanum*. The reduced egg develops into a new plant without fertilisation.

(ii) By male parthenogenesis or androgenesis. The sperm nucleus develops into an embryo.

(iii) Of the four megaspores derived from a single megaspore mother cell, more than one develop into an embryo. One may be due to fertilisation and other parthenogenetically as in *Oryza sativa*.

(iv) The reduced cell of the embryo sac, such as synergid or antipodal cell, may develop into an embryo parthenogenetically.

(v) Egg may develop into embryo parthenogenetically by receiving parthenogenetic stimulation. Male nucleus fuses with the polar nuclei giving rise to tetraploid endosperm. It may provide stimulation to egg as in *Triticum compactum*.

(vi) Parthenogenetic stimulation may develop by dusting the X-rayed pollen on stigma. It was noticed occasionally in *Nicotiana rustica* and *Triticum monococcum*.

(vii) Pollen from widely related species may provide stimulation without actually participating in fertilisation as in *Datura ferox* and *D. stramonium*. The pollens of former stimulate parthenogenesis in latter.

(viii) Parthenogenesis may be stimulated by applying low temperature.

Haploid plants are less vigorous and highly sterile. Morphologically they are reduced replicas of diploid forms. Meiosis in them is quite irregular and the absence of homology leads to non-pairing between chromosomes. No regular equatorial plate is formed at first division and therefore, chromosomes lie scattered on the spindle. In haploid plant of rice (*O. sativa*), homologous pairing was absent and 12 univalent chromosomes scattered in diakinesis were observed and at anaphase I, the chromosomes separated and grouped randomly as 11-1, 10-2, 9-3, 8-4, 7-5, 6-6 etc. Sometimes spindle becomes crescent-shaped resulting in the formation of three nuclei.

Haploids are commonly sterile but they produce new progenies under exceptional circumstances when either dyads are formed with 0-*n* distribution (0-12), or heterotypic division is omitted or univalents may divide or monads with *n* or 2*n* chromosomes are formed.

Haploids may be of two kinds—(1) monohaploids, (2) polyhaploids. When an haploid arises from diploid organism, its called monohaploids or monoploids but if from polyploids called polyhaploids. These polyhaploids may be allohaploids or pseudohaploids. Allohaploids arise from allopolyhaploids and contain two haploid sets of chromosomes, one each from the parental species. While pseudohaploids are haploids from tetraploids and they are, therefore, diploids in true sense.

Haploids are of little practical utility except for their biological importance and from plant breeding point of view. They would breed true after self fertilisation. Diploids produced from haploids will possess a completely homozygous set. They would be homozygous for all their genes.

Thus, in breeding experiments synchronised results may be obtained. Artificially induced haploids from complex polyploids may be used for economic breeding. Haploids are also useful in determining the genetics of particular characters by studying random segregation during haploid meiosis.

AUTOPOLYPLOIDY

Autopolyploids are obtained when the same sets of chromosomes of a genome are increased. In general, autopolyploids show the vigorous growth. Their leaves are broader, darker green and often with crinkled surface. Floral parts, fruits and seeds are comparatively bigger than diploids. They are infrequent in nature but are evidently known from the seedlings of Graminae and polyembryonic seedlings of *Citrus* plants.

Since they have more than two sets of homologous chromosomes, the pairing process becomes complex. All the homologous complements come closer but at one point the pairing occurs in between two chromosomes only. They are referred as trivalent, tetravalent, quinivalent etc. to designate the association of two, three or four homologous chromosomes held together by chiasmata. If pairing occurs in between the chromosomes of same parental origin, the phenomenon is termed as **autosyndesis**. During metaphase, the disjunction (separation of chromosomes) becomes irregular in multivalent chromosomes and as a result abnormality and certain amount of sterility may arise. So the reduced fertility of autopolyploids is due to the presence of multivalency and non-disjunction.

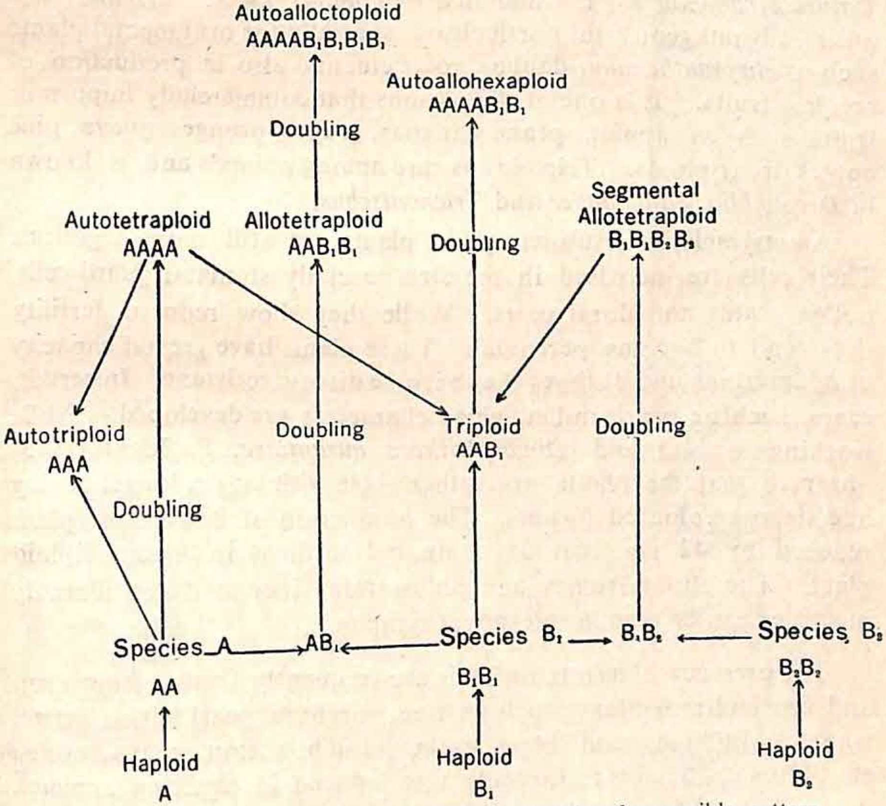


Fig. 12.2: Different types of polyploids elucidating the possible pattern of their origin.

AUTOTRIPLOIDS : Gates (1908) for the first time reported triploidy in *Oenothera*. Since then it has been recorded in many other plants. They may arise by any one of the following ways.

(i) When during sporogenesis, reduction of chromosome number fails, diploid gametes are formed. On their fertilisation with haploid gametes, triploids are formed.

(ii) When diploid gamete produced from tetraploid plant fertilises the haploid gamete, triploid is formed.

(iii) When tetraploid plants are crossed with diploid plants.

(iv) When haploid egg is fertilised twice (dispermy) by two haploid males, triploid is formed.

Triploids are more vigorous than haploids. They are more leafy and tend to become perennial. The plants are highly sterile. The fertility as determined by seed germination in *Zea mays* is 5·3%, in *Datura stramonium* 4·1% and in *Pyrus malus* 1·4%. Triploids are quite advantageous in horticulture specially for ornamental plants such as *chrysanthemum*, dahlias, roses etc. and also in production of seedless fruits. It is one of the reasons that commercially important fruits such as apples, pears, bananas, grapes, oranges, guava, pine apples are triploids. Triploidy is rare among animals and is known in *Drosophila*, *Salamander* and *Trichonischus*.

Autotetraploids : Autotetraploid plants are still more vigorous. Their cells are increased in the size specially stomatal guard cells, pollen grains and floral parts. While they show reduced fertility they tend to become perennial. These plants have greater capacity of adaptations and at times they become disease resistant. In certain cases, such as pearl millet, gigas characters are developed. While working on tetraploid *Dimorphotheca aurantiaca*, S. Rizvi (1973) observed that the plants are rather gigas with larger, longer lasting and deeper coloured flowers. The production of flowers per plant reduced by 42 per cent as compared to those in normal diploid plant. The chromosomes and pollengrains increased considerably in size as can be seen in microphotographs.

The presence of autotetraploids are frequently known from crop and horticultural plants such as rice, sorghum, pearl millet, maize, wheat, chilli, red gram, black gram, lab-lab, cotton, guava, coffee etc. When artificially tetraploidy was induced in *capsicum annuum*, it was found that number of seeds per fruit was reduced while the size of pollen was increased. Several pollens were incompatible. The tetraploid plants are of great economic importance and because of their breeding possibility, the autotetraploids are now frequently produced artificially.

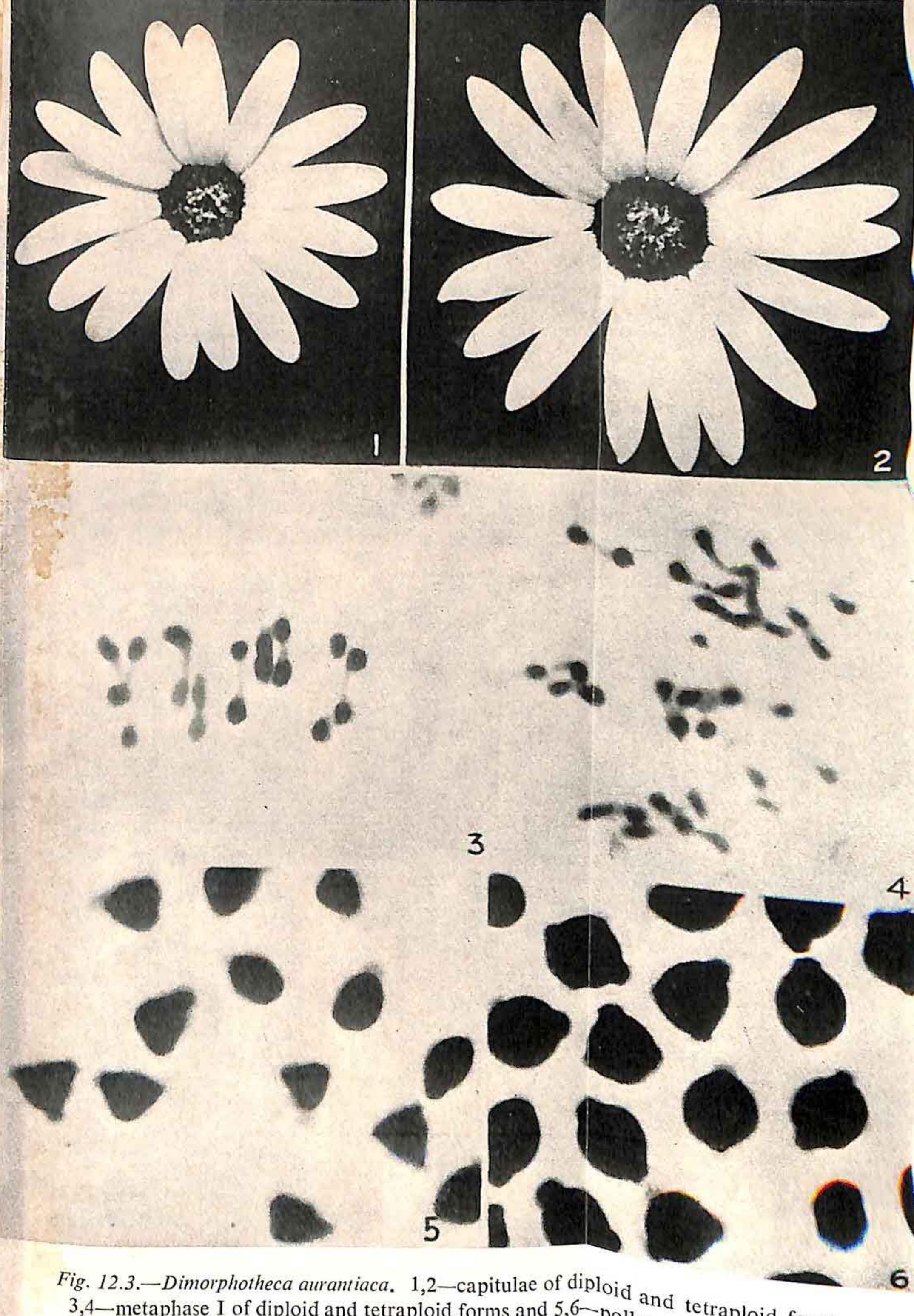


Fig. 12.3.—Dimorphotheca aurantiaca. 1,2—capitulae of diploid and tetraploid forms, 3,4—metaphase I of diploid and tetraploid forms and 5,6—pollen grains in diploid and tetraploid forms. (*Courtesy—S. Rizvi, N.B.G.*, Lucknow. 1973).

Autotetraploids are often produced by somatic doubling which generally happens by the failure of mitotic division in zygote. Somatic doubling also occurs from buds initiated by callus tissues. Occasionally by the fusion of diploid gametes, tetraploid plants are formed.

ALLOPOLYPLOIDY

Allopolyploidy arises by the hybridisation of species which may be intrageneric or intergeneric and it brings into the hybrid a qualitative change. Allopolyploids are quite common in nature and majority of the naturally occurring polyploids is probably allopolyploids. Quite many species of maize, potato, sugar cane, banana, tobacco, apple, pear, rose, dahlia and garden lillies are allopolyploids. One of the most fascinating outcomes of allopolyploidy is the production of a new cereal *Triticale* obtained by crossing of rye with wheat.

Polyploidy has been the best means to restore fertility in the sterile hybrids. When two genetically different species are crossed, the hybrids show only univalent at meiosis with the result sterility is produced. This defect could easily be overcome by duplication of chromosomes in hybrids. Such diploids are called amphidiploids.

The classical and the most suitable example of the amphidiploidy is the production of *Raphanobrassica* by the intrageneric cross. Karpechenko (1928) made a cross between *Raphanus sativum* (radish) and *Brassica oleracea* and produced a new genus *Raphanobrassica*. Both parent plants were diploid each with $2n = 18$. The gametes produced contain chromosomal complement $n=9$ and, therefore, F_1 hybrid had $2n = 18$, i.e., 9 chromosomes each from

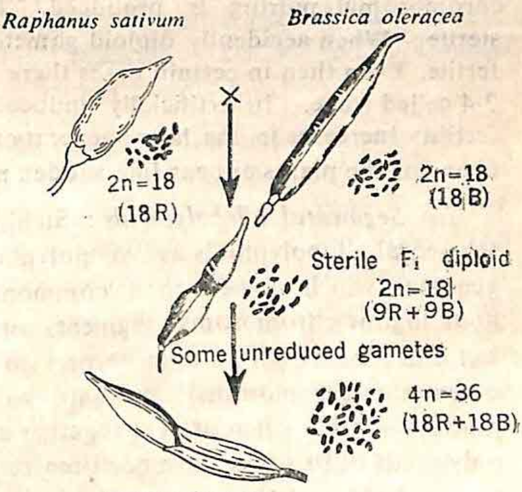


Fig. 12.4 : Diagram showing artificial synthesis of *Raphanobrassica*

Raphanus and *Brassica*. The F_1 was sterile obviously due to lack of homology between the chromosomes of two different parents and the normal gametes could not be formed.

Luckily in his experiments few spore mother cells did not undergo reduction division and produced gametes with $n=18$. The fusion of two such gametes led to the formation of F_2 individuals with 36 chromosomes, i.e., 18 pairs of homologous chromosomes. So these allotetraploids (amphidiploids) became fully fertile and possessed the characters of both the species. They bred true in future course of generation.

The allopolyploids can be further classified and three different categories can be recognised—the true or genomic allopolyploids, segmental allopolyploids and autoallopolyploids.

(i) *True or genomic allopolyploids* : Some well known examples of induced true allopolyploids are the species of *Nicotiana, Raphanobrassica, Secale, Triticum. Triticale* etc., while those in nature are found certain species of *Rubus, Nicotiana rustica, Brassica napus, B. juncea* etc. These are typical allopolyploids as they are derived from the hybridisation between two or more distantly related species. Their chromosomal complements differ so much that the pairing is not possible in F_1 hybrids. Only a small number of chromosomal pairing is produced. Therefore, these plants are sterile. When accidently diploid gametes are produced they become fertile. Even then in certain cases there is abortion of embryo sac at 2-4 celled stage. In artificially induced polyploids, the degree of fertility increases in the later generations. Because of the sudden changes, the plants appear like sudden mutants.

(ii) *Segmental allopolyploids* : Stebin has defined precisely the segmental allopolyploids as "a polyploid containing two pairs of genomes which possess in a common a considerable number of homologous chromosomal segments or even whole chromosomes, but differ from each other in respect to a sufficiently large number of genes or chromosomes segments, so that the different genomes produce sterility when present together at the diploid level. Natural polyploids of this type have not been recognised in many instances. In morphological characters and in meiosis they stimulate autopolyploids. Their real nature remains uncertain until both of its parents have been identified." *Solanum tuberosum* and *Sorghum halepense* are considered as natural segmental allopolyploids while artificially induced ones have been found in the species of *Tradescantia*, *Nicotiana*, *Delphinium*, *Paeonia*, *Lotus* etc. and in *Crepis foetida-rubra* and *Allium sepa-fistulosum*.

(iii) *Autoallopolyploids* : Autoallopolyploidy are confined to hexaploidy and upwards. These contain a characteristic combination of allopolyploid and the segmental-allopolyploid. Common examples of hexaploids are found in *Helianthus tuberosus*, *Phleum pratense* and *Solanum nigrum*. They are supposed to contain genomic formula *AAABBB*. In octaploid, the genomic formula will be *AAAA BBBB* which is known in *Nicotiana tobaccum* and *Fragaria*.

ANEUPLOIDY (Heteroploidy)

Heteroploids contain irregular number of chromosomes made up of a part of genome. They are not exact multiple of the diploid or haploid rather one or more chromosomes get added or become deficient to the normal sets of chromosome. So they may be of two types—hypoploidy and hyperploidy. The nucleus of hyperploidy receives one or more chromosomes extra while hypoploidy has one or more chromosomes less than normal. In most cases, aneuploidy arises due to non-disjunction of chromosome during cell division. As a result, gametes formed have either $n+1$ or $n-1$ genome. These on fusion with normal gamete (n) produce F_1 either with $2n+1$ or $2n-1$ constitution. The plants $2n+1$ are called trisomics while $2n-1$ monosomics. Trisomy has been reported in *Datura* and *Drosophila* and monosomy in *Nicotiana*, *Triticum vulgare* etc.

TRISOMIC ANEUPLOIDY : It has been reported in *Datura stramonium*, *Drosophila melanogaster* and *Zea mays*. Such plants contain an additional homologous chromosome which interferes in pairing during cell division. Since only two homologous chromosomes can pair at any position, several arrangements are possible when three homologues are involved in pairing. A trivalent configuration will result from an association of three homologous chromosomes.

A good example of trisomy is the Triplo IV of *Drosophila* in which chromosome IV is present three times. The fly develops narrow pointed wings, coarse bristles and smooth eyes. When such trisomics are mated with normal, the ratio between normal and typical individual comes to 2 : 1 or 5 : 1 depending upon gametes viability.

SECONDARY TRISOMICS : Secondary trisomics arise from primary trisomics which contain three chromosomes, two normal and one extra which has two similar ends. As a result they have a closed ring of three chromosomes during meiosis in contrast to primary trisomics. Because bivalent and univalent chromosomes act abnormally, the changed phenotypes are produced. Secondary and more complex trisomics have been found in Datura. Out of 24 possible secondary trisomics in *Datura*, 12 have been recognised.

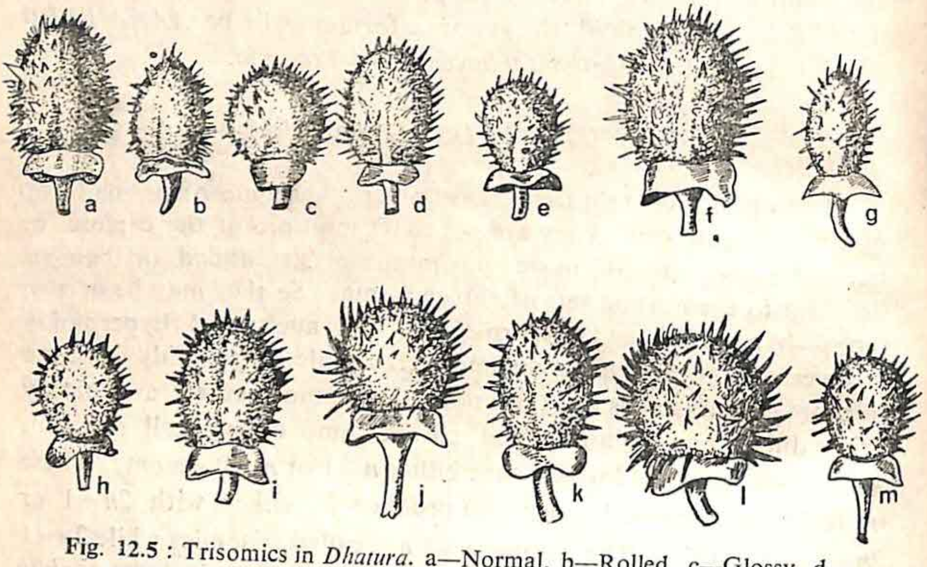


Fig. 12.5 : Trisomics in *Dhatura*. a—Normal, b—Rolled, c—Glossy, d—Buckling, e—Elongate, f—Echinus, g—Cocklebur, h—Microcarpic, i—Reduced, j—Poinsettia, k—Spinach, l—Globe and m—Ilex.

MONOSOMIC ANEUPLOIDY : They arise by the fusion of a normal gamete and a gamete with $n-1$ constitution. The zygote then has $2n-1$ chromosomal set. Such individuals would have one chromosome only once and not in duplicate (homologous pair).

Monosomic plants are known in *Nicotiana tobaccum* and in hexaploid wheat-*Triticum vulgare*. Twenty out of twenty four possible monosomics in *N. tobaccum* and all possible 21 types of monosomics in *T. vulgare* have been observed and studied.

NULLISOMIC ANEUPLOIDY : In nullisomics, a pair of homologous chromosomes is absent. These may arise by selfing of monosomics but are produced in poor percentage 0·9 to 10·3%. Nullisomics differ remarkably from their parents. Besides, they are of valuable academic interest in studying the biology of genetic factor.

INDUCTION OF POLYPLOIDY

To the plant breeders and geneticists, it has been of great interest to induce the polyploidy since it brings many significant morphological changes in the plants. Polyploidy has been helpful in producing new strains, varieties and species of economically important plants.

Aberration in cell division leads to develop polyploidy. Commonly the doubling of the chromosomes occurs during the failure of formation of cell wall at the time of meiotic division of nucleus and as a result, the shoot developed from such cells becomes tetraploid. Polyploidy may also be achieved by inhibiting the spindle formation. The sister chromatids will fail to separate and consequently the chromosome number is double. Besides, because of failure of first or second or both divisions in meiosis (restitutional nuclear division) or failure of homotypic division after reduction division or double divisions of chromosomes, polyploid gametes may be formed i.e., to say aberration during meiosis may lead to the formation of polyploid gametes by fusion of which polyploid plants are produced.

The various means employed for the production of autopolyploidy are

(i) Heat and cold shocks to flowers at the time of first division of zygote.

(ii) Use of certain chemicals (chemical induction) such as Acenaphthene, Colchicine, Coumarine, IAA, Granosan, Veratrine sulphate, Cevadine etc.

(iii) Inducing wound or injury to promote callus tissues and then inducing bud formation from it.

(iv) X-rays irradiation.

The production of *Raphanobrassica* has been the first classical example of induced allopolyploidy (Karpechenko, 1927). Since then polyploidy has been induced successfully in many plants and animals. The most promising results have been obtained with colchicine treatment. Colchicine is now widely used. The common example of colchicine induced polyploidy are seen in *Capsicum*, *Gossypium*, *Cicer*, *Zea mays*, *Tradescantia*, *Petunia*, *Antirrhinum*, *Portulaca*, *Datura*, *Cucurbita* etc. The effect of colchicine was observed by Blakeslee, Avery and Nebel (1937).

Colchicine is an alkaloid obtained from the plant *Colchicine autumnale*. It grows frequently in the temperate regions particularly that of Medeterranean and Central Asia. The alkaloid is also obtained from *C. lupium* (a species growing in W. Himalayas) and *Gloriosa superba* (Parthasarthy, 1941). Low concentrations (0·05, 0·25, 0·5, 1% or so) of colchicine is applied in the region of actively dividing cells. The techniques for its application may be as follows.

(a) The twig may be immersed in colchicine solution of various concentrations.

(b) Solution of colchicine in agar agar may be applied.

(c) Drops of colchicine may be placed on the flowering bud at proper interval.

(d) Different concentrations of solution may be spread.

(e) It may be applied as a paste in lanolin or gum.

(f) Seeds are soaked in aqueous solution of colchicine for 24 hours or so.

When colchicine becomes effective, the general symptoms such as immediate arrest of growth, increase of stomatal size, delayed flowering, bigger leaves and flowers, increase of cotyledonary node (in seed treatment) etc. are seen.

Colchicine brings about doubling of chromosome by inhibiting spindle formations during cell divisions. Such effects can be particularly visualised on the germinal layers which ultimately spreads to the outer tissues such as reproductive ones. When colchicine was applied on cotton plant (*Gossypium anomalum*), the leaves become larger, darker in colour and leathery in texture, leaf lobes overlapped and twisted, stomata, bracts, pollen grains, balls and seeds increase in size. The plants were relatively more fertile.

The chemicals frequently used for inducing polyploidy are described earlier. However, the role of *IAA* (Indole acetic acid) in inducing polyploidy was found in a way so as to stimulate the rapid cell division in otherwise infrequently dividing regions (Huskin and Steinitz).

POLYPLOIDY IN ANIMALS

Polyploidy is well known among plants especially flowering ones but amongst animals, the cases of polyploidy are very rare. It is neither because of that the doubling of chromosome does not occur during cell division nor the individuals fail to live. Triploid and tetraploid *Drosophilae* are known, so is true in many other cases. Even then the polyploidy is rare among animals.

Explaining the reasons for the rare occurrence of polyploidy as compared to the plants, it is seen that animals usually have two highly distinct sexes which are differentiated by means of a process involving the diploid mechanism of segregation and combination. Whereas plants are usually hermaphrodite, in animals the diploid sex determining mechanism and bisexuality act in many ways to hinder the establishment of any polyploid race. Following may be the reasons as proposed by Muller (1925).

(i) Triploids are intermediate between diploid and tetraploid and, therefore, in bisexual organisms the proportion of the sex producing genes (XX or XY) normal to heterozygous sex cannot exist in triploids. And hence, triploids or the other may fail to be fertile. Bridge (1921), however, supposed that $3X$ triploid *Drosophila* is fertile and is superfemale. All other triploid forms were sterile. It means that fertile triploids are possible but only females. They will have no triploid male with which to breed.

(ii) Occasionally tetraploids may arise without interbreeding or selfing of triploids. The reasons may be the same as in plants e.g., the failure of disjunction of chromosome during cell division may lead to the formation of diploid gametes and so on. If somehow the tetraploid animals arise, it will have to breed with diploids again and triploids will then result. Theoretically with rare coincidence tetraploid may breed with another tetraploid or triploid to establish a tetraploid race, yet, it will be far more difficult to maintain and to avoid their breedings with diploids. Genetic isolation of them is to be maintained.

(iii) The established tetraploid race if, however, produced would still be at a reproductive disadvantage as compared with diploids under conditions of natural struggle of existence. They will be handicapped in the sex determining mechanism and gametes formed would result in $1XX : 4XY : 1YY$ gamete ratio. There will be random synapsis and certainly the XY gametes of the tetraploid would be in the great majority. When XY gametes will fertilise XX egg of tetraploid, sterile zygote will be produced which will have interproportion of sex-producing genes normal to neither male nor to female.

(iv) Besides, animals are specialised creation of nature. Most animals would have a relatively high fixity in number of genes. During course of evolution, the regions of chromatin would have become increasingly differentiated from each other and incapable of carrying on necessary functions performed by other regions. Under such circumstances, any little action producing inactivation in the region, would arise. In plants, tetraploidy arose fairly recent and, therefore, may be more capable to act in substitution for them than animals.

Any way, many polyploid animals have been reported. Harvey (1920) has mentioned to this extent that polyploidy of some sort might have played role in evolution of animals. It is expected, considering above facts, that polyploidy in the hermaphrodite organisms as in plants may be possible. It is true also. In *Hirudinea*, number 8 and 16 have been found together with other in between. The genus *Mesostoma* (of rhabdocoela) is known to have 2, 4, 5, 6 and 8 chromosomal complements. Therefore, it may be visualised that 4 and 8 chromosome containing species may be tetraploid or octaploid respectively. Other cases of polyploidy have also been reported such as in *Neodiprion* and *Diprion*, 7 chromosomes are present in male and 14 in female. The male develops parthenogenetically as in other Hymenoptera. However, *D. simile* has 24 chromosomes in female and 14 in male. Smith considered this species as allotetraploid but White (1924) on the precluded quadrivalent formation considers it a case of autotetraploid. White stated that no polyploid race among animals has been established in the vertebrate group with the exception of golden hamster.

POLYPLOIDY IN EVOLUTION

While going through the list of polyploidy among plants, it becomes clear that it is quite common and that the degree of polyploidy varies in the different genera, species and subspecies. Polyploids occupy a much wider area than diploids in nature. They have spread into environmental zones with relatively much success.

Autopolyploids frequently develop sterility due to multivalent chromosomal association specially in long chromosomes but those with short chromosomes form bivalents due to pairing conditions. In many cases the autoploidy show immediate sterility but in later generations fertility increases due to elimination of chromosomal irregularities. Amphidiploidy in such cases has frequently helped overcoming these difficulties. The stabilisation of the phenomenon involves the rearrangement of the chromosomal affinity so as to reduce multivalent formation. In cases where two genomes differ in small segments, the selection would be in favour of elemination of these segments and evolution would be in the direction of autopolyploidy while in case of long differences, selection would be favoured by means of mutation, chromosomal rearrangement etc. to avoid multivalent formation.

Under natural conditions, amphidiploids are less stable and they are, therefore, subjected to certain changes before they form stable species in nature, e.g., in *N. tobaccum* the elimination of duplications is one of the important steps. The progeny may thus become secondarily balanced, i.e., the form may have a new basic number as in Pomoideae. Similarly, Darlington and Mather have shown that apple is fundamentally a complex polyploid, i.e. a combination of partly hexaploid and partly tetraploid.

Certain plants obtain extra chromosomal compliment and thereby confer certain advantages to the species which are lacking in diploid forms. In autotetraploids, two extra sets of genes are available for mutation without seriously affecting the existing characteristics. The deleterious effects are immediately reflected in the progenies of diploid while in polyploids the deleterious effects are buffered by the presence of other normal allelomorphic genes. Favourable mutation is selected in such cases. Therefore, polyploids are important from the point of view of evolution and during the course of evolution tetraploids or other polyploids have been established and have begun to behave like diploids.

Polyploidy increases viability and, therefore, become asset for making it possible for a number of sub-species, microspecies etc. to come into existence, e.g., Rosa-Canina complex.

Polyploid forms develop perennial habit and effective means o. vegetative reproduction. Plants become more capable of overcoming the difficulties of sexual sterility and environmental extremes. They are relatively more successful under new ecological conditions. Stebbins states that "Polyploidy may be looked upon as a process which is most effective as a means of enabling species groups which have reached a certain stage of depletion of their biotypes, and of sharp divergence of specific entities which arise suddenly. It is much less important in stable environments and in diploid species which are still widely distributed and rich in ecotype differentiation." Muntzing (1936) has extended his opinion to the extent that perennial forms have been evolved from annual forms through polyploidy.

POLYPLOIDY AND AGRICULTURE

Polyploidy has been immensely important in maintaining the desired crop plants and others. Many of them known for their established significance are listed here.

Table 12. II. Showing some polyploid forms

Name of the plant	*No. of chromosomes*	*Name of the plant*	*No. of chromosomes*
Wheat $n=7$		**Cotton** $n=13$	
Triticum monococcum	14	*Gossypium arboreum*	26
T. dicoccum	28 (Tetraploid)	*G. hirsutum*	52
T. durum	28	**Trifolium** $n=8$	
T. polonicum	28	*Trifolium hybridum*	16
T. turgidum	28	*T. repens*	32
T. dicoccoides	28	*T. medium*	80
		Medicago $n=8$	
T. vulgare	42 (Hexaploid)	*Medicago hispida*	16
T. compactum	42	*M. sativa*	32
T. spelta	42		
Sorghum $n=5$		**Avena** $n=7$	
Sorghum versicolor	10	*Avena brevis*	14
S. vulgare	20	*A. abyssinica*	28
S. halepense	40	*A. sativa*	56

While attempts to use polyploidy in plant breeding have not yet met any great success, the selection and Mendelian recombination of genes have been the efficient means in it. The cause of its unsuccess may be partly due to want of proper techniques and partly due to certain factors advanced by polyploidy. There is always partial sterility to be contended within species hybrids. The more homozygous the parents, the greater will be the hybrid sterility. Self incompatibity also brings difficulties in successful breeding. Besides, the increase in chromosome number brings disadvantage to plants unless species are accustomed to maintain high number.

However, allopolyploidy has been of greater importance to agriculture than autopolyploidy. The breeder can achieve the improved desirable economic plants from allopolyploids and by well planned back crossing, it will be possible to utilise the amphidiploids in transferring desirable genes and raising new types of plants. Amphidiploids form a bridge between the species which do not cross, e.g., *Raphanobrassica* (example discussed earlier).

Some other examples of polyploidy are discussed herewith.

1. TRITICALE : *Triticale* is the first hybrid grain fully synthesised by man. It is a *Triticum Secale cereale* hybrid (*Triticale*). The hybrid was sterile but the fertility was restored by producing its amphidiploid using colchicine treatment. *Triticale* plants are stiff straw and deep rooted and they do not lodge at high fertiliser application. These thrive well under low moisture condition and give a good yield also. A normal crop of *Triticale* with dwarf varieties can give about 70 quintals grain/hectare. Even in sandy and poor soils without irrigation it may yield up to 24 quintals per hectare. Grain quality of *Triticale* is also good as it has 16 per cent protein as against 12 per cent in wheat and 8 per cent in rye. In India, the cultivation of this crop is under trial in M.P., Bundelkhand, Agra, J. L. Nehru University, Pantnagar and IARI, Delhi.

2. WHEAT : There is yet another good example of evolution by amphidiploidy which explains the origin of common bread wheat. The fig. no. 12.6 explains it in a summarised way. It, however, visualises that a rare hybridisation between distantly related strains can some time be stabilised by a subsequent doubling of the entire complement. The homologous chromosomes from rather different original parents no longer pair in meiosis. Occasionally, pairing may be limited to the closely related homologues of each parental complements. However, the chart shows that regularity of meiosis and fertility of the new hybrids are assured if amphidiploidy is called on. Based on this fact, the chart summarises the origin of common bread wheat (*Triticum vulgare*) in which the role of unreduced gametes that produce amphidiploid have been vital.

A perusal of chart indicates that *T. vulgare* is hexaploid with $2n=42$ of allopolyploid nature. It has been derived from three diploid species (1) *Triticum aegilopoides* (A), (2) *Aegilops speltoides* ? (B) and (3) *Aegilops squarrosa* (D). Recent studies have indicated that three species are not much different from one another and is believed that they were derived from a common ancestor. If it is taken into account, the hexaploid wheat is an autopolyploid form

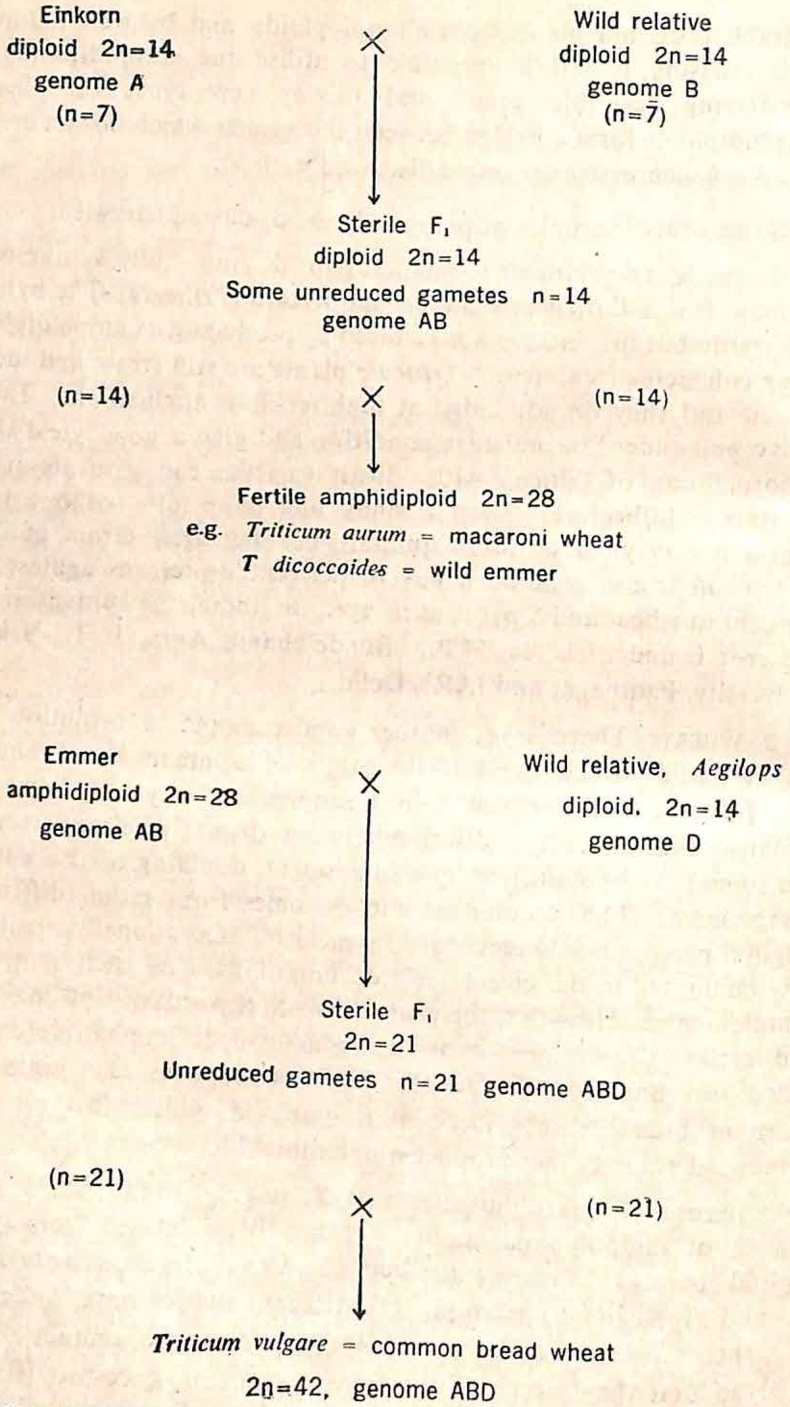


Fig. 12.6 : Origin of common bread wheat, a case of evolution by amphidiploidy.

and not an allopolyploid form. It may however be considered as segmental allopolyploid.

T. spelta is an artificially synthesised hexaploid wheat developed from a cross between emmer wheat and goat grass (*Aegilops squarrosa*) by E. S. Mc Fadden, E. R. Sears and H. Kihara, 1946. The method is described here.

Triticum dicoccoides × *Aegilops squarrosa*
(Tetraploid wheat)
genome – AABB DD
$2n = 28$ $2n = 14$

↓

ABD
$2n = 21$ (Univalent triploid hybrid)

↓ Colchicine

AABBDD
$2n = 42$ (Synthesised hexaploid wheat)
Triticum spelta

3. GOSSYPIUM (Cotton) : *Gossypium hirsutum* is a tetraploid new world cotton. This tetraploid form is in fact an amphidiploid form originated from two species—*G. herbaceum* ($2n=26$) and *G. raimondii* ($2n=26$). *G. herbaceum* has 13 pairs of small chromosomes and *G. raimondii* has 13 pairs of large chromosomes. The hybrid treated with colchicine produced new world cotton containing 26 pairs of chromosomes (13 small + 13 large). The new world cotton is now widely grown.

Similarly, *G. anomalum* ($n=13$), a new world wild cotton was crossed with cultivated old world cotton—*G. arboreum* ($n=13$). The hybrid was sterile but when treated with colchicine amphidiploidy was produced which crossed freely with *G. hirsutum*. Yet another amphidiploids were produced by Amin from a cross between *G. anomalum* & *G. davidsonii*, and *G. anomalum* & *G. herbaceum*. These were crossable with American types. This way desirable characters could be brought together in a form.

4. NICOTIANA : *Nicotiana tobaccum* is susceptible to downy mildew and blue mould whereas Australian allied species are resistant to them. Since both these species were genetically different, hybrids were sterile. Sterility in F_1 is overcome by chromosome doubling.

N. glauca contains an alkaloid *anabasine* which is more active than nicotine. Its allotetraploid form shows high percentage of same and plants grew more vigorously.

GENERAL QUESTIONS

1. What is polyploidy ? Write an essay on it.
2. What are different kinds of polyploids ? Write critically.
3. What are haploids ? How meiosis occur in these plants ? Give their significance.
4. Why polyploidy is rare among animals than in plants ?
5. Write an essay on induction of polyploidy.
6. "Polyploidy has played a greater role in evolution." Discuss critically with suitable examples.
7. Write short notes on (i) Euploidy, (ii) Aneuploidy, (iii) Nullisomics, (iv) Autopolyploidy, (v) Triticale and (vi) Amphidiploids.

■ ■ ■

CHAPTER 13

SEX DETERMINATION AND SEX REVERSAL

While asexual reproduction is widely present in plants only, sexual reproduction is of common occurrence in both animals and plants which in higher organisms is well advanced type and biparental, i.e., male and female individuals are separate. Both male and female individuals in animals differ not only in the occurrence of two kinds of sex-organs but also in possessing the secondary sexual characters whereas in plants male and female do not differ except in their floral characters. In plants, monoecism is also common and may bear bisexual flowers. However, dioecious organisms make the problem more interesting to visualise the reasons or factors responsible for determining the sex whether a particular individual would be a male or female. This aspect, in fact, is the actual problem of sex determination. Darlington and Mather (1949) stated that "Sex determination is the process by which a spore (haploid) or egg (x) develops the properties of one or other sex. This term should not be confused with sex differentiation. Sex differentiation is the later development of anatomical and other differentiations exhibiting unmistakable characteristics of one or other."

BRIEF HISTORY : The problem of sex determination has been of prehistoric interest. Earlier the old men emphasised on the importance of external environment in determining the sex and so on. Several points were advanced by Hippocrates, Thury, Garen etc. in this relation such as relative vigour of parents, their relative age, the type of nutrition of mother, season of mating, the ripeness of egg, the particular ovary or the testes producing the gametes etc. The more vigorous parent offers his or her sex to the offspring or if egg is fertilised soon after ovulation it gives rise to female while it produces male if it remains in the oviduct for some time before fertilisation or if the germ cell is produced from right ovary it will develop into male and if from left will be female etc. These theories were not based on any scientific fact. Therefore, the investigators finally turned their attention in exploring some internal mechanism in sex determination and found several true explanations. Three possibilities of sex determination based on embryological studies were advanced.

(i) *Progamic*—Sex is determined before the fertilisation.

(ii) *Syngamic*— It is determined at the time of fertilisation.

(iii) *Epigamic*—It is determined after the zygote has been formed.

Syngamic sex determination has been popularly considered by modern workers. Of the many theories proposed a few are discussed here.

1. Chromosomal Theory of Sex Determination

Almost at the time of rediscovery of Mendel's law, McClung (1902) showed the formation of two kinds of sperms produced in equal number in certain insects. One of which has extra accessory chromosome, not present in the other. He suggested that this chromosomal difference in male gametes is in some way related with sex determination and that the egg fertilised by one type of male gamete produces male and by the other type produces female. These chromosomes when became firmly distinguished from other were referred as sex chromosomes and others which were equally present in both sexes were autosomes. Thus, according to this theory there will be two types of chromosomes present in each individual— (i) autosomes and (ii) sex chromosomes.

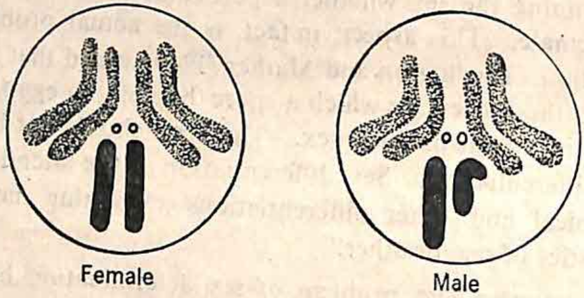


Fig. 13.1 : Chromosomal set of male and female *Drosophiila*. Autosomes are shown dotted while sex chromosomes completely black. *X* chromosomes are solid rod shaped while *Y* is curved, small and inverted comma-like. The two small spheres in centre indicate diploid nature of organisms.

In sex chromosomes, two different types were observed. The female individual has $2x$ chromosomes while male has one x and one peculiar chromosome called y chromosome. The female may therefore be described as xx individual and male as xy type. The female on gametogenesis produces only one type of egg, each containing an x chromosome while male produces two kinds of sperms—half of the sperms contain x chromosome while rest half y chromosome, i.e., males are heterogametic. The fertilisation of the x egg with x sperm produces an xx individual which develops into female while the fertilisation of x egg with y containing sperm produces male individual. The sex is, thus, determined at the time of fertilisation.

Parents	Female	×	Male	
	xx		xy	
Gametes →	x		x y	(two types of gametes; 80% contain x and other 50%y)
Fertilisation and Offsprings	(if by x of male) ↓ xx (female)		(If by y of male) ↓ xy (male)	

In several other examples such as moth, birds and fishes, the female contains xy chromosomal complement and male xx. These are opposite type of cases. In both these cases, the sex is determined by the chromosomal combination of mating gametes.

It then becomes evident that in these cases, sex determination seems to be dependent on the presence of one of the two sex chromosomes. This theory is sometimes called "*Heterogamosis*" as reported by Correns which presumes that one gamete is heterozgyous and other homozygous. Since this theory has cytological basis, it has been widely supported. In *Drosophila*, man and most of the plants, males are heterozygous while in birds, moths, butterflies, cuddies etc. female heterozygosity is reported. The case of grasshopper is still different where male has only one sex chromosome, thus producing two types of sperms that differ from each other by a single chromosome called x chromosome. Female has xx chromosomal complement while male xo.

Sex chromosomes have been variously named such as x and y chromosomes, z and w chromosomes, odd chromosomes, idiochromosomes, heterosomes or allosomes and they are supposed to have genes for sex determination.

2. Metabolic Theory of Sex Determination

While working on doves, Riddle concluded that sex determination is fundamentally based on physiological approaches rather than cytological ones and stated that "sex determination is conditioned by the degree of metabolism involved and is indicated by an increase in the rate of oxidation, larger water content and less protein content." He further suggested that "the chromosomal constitution is not sufficient cause of sex. It is but a sign or index and possibly an assistance in the normal maintenance of what is essential, namely, two different metabolic levels." He even accepts that sex is not determined by genes or chromosomes.

3. Nutritional Theory of Sex Determination

Propounded by Sharp (1934), this theory visualises that the sex determination depends upon constitution of the protoplasm concerned as well as upon the condition in which this protoplasm is compelled to react such as external and internal changes in habitat, nutrition, influence of hormones, parasitism, disease or other.

4. Quantitative Theory of Sex Determination

The occurrence of intersexes in nature and the production of intermediate forms between two extremes of sex led Gold Schmidt to postulate quantitative theory that the sex determination depends on the quantitative dominance or balance of parents.

5. Genic Balance Theory of Sex Determination

None of the arguments or theories proposed earlier could satisfactorily explain the mechanism of sex determination. However, it could be concluded that Y chromosome is not very necessary in this mechanism. Suddenly while working on *Drosophila*, Bridges (1922) obtained triploid forms containing three sets of chromosomes, i.e., $3A : 3x$ (3 sets of autosomes and 3 sets of sex chromosomes). These triploid females were normal females. He made a cross between triploid females and diploid males $(2A + xy)$ and obtained normal diploid males, triploid females, intersexes, super males and super females, all phenotypically different forms. The intersex means such individuals which are in between male and female and are sterile.

The result proved that chromosomal constitution of these forms has a definite role in the mechanism of sex determination.

The presence of triploid intersex led him to presume that autosomes also carry factors for sex determination. Based on this fact he propounded a theory called *Genic Balance Theory of Sex Determination*. According to this theory sex is determined by the ratio present between the number of *x* chromosome and chromosomal set or autosome set i.e., *x/A* ratio. *x* chromosome carries female potentiality while autosomes male. Thus, both genes participate in functioning and the balance becomes the determining factor of sex. The result of the experiment is shown in the table (Table 13.I).

Table 13.I. Different sexual types in *D. melanogaster*

Forms of individual	*x-chromosome*	*Set of autosomes (n)*	*Sex index ratio x/A*	*Sex type*
Diploid	3	2	1·5	Superfemale (metafemale)
Triploid	4	3	1·3	,,
Haploid	1	1	1	Female
Diploid	2	2	1	,,
Triploid	3	3	1	,,
Tetraploid	4	4	1	,,
Triploid	2	3	0·67	Intersex
Tetraploid	3	4	0·75	,,
Diploid	1	2	0·5	Male
Tetraploid	2	4	0·5	,,
Triploid*	1	3	0·33	Supermale (metamale)

The table depicts that if the sex index ratio is one or above, the sex of offspring will be female, if in between ·67 and ·75 intersex and if ·5 or below, it will be male.

The theory enjoys the support of Goldschmidt (1955) who states that "if the female sex is located in the *x* chromosome, the male is outside the sex chromosome which may be then either in autosomes or in *y* chromosome or in both. The male sex determiners are, therefore, equally present in both sexes, but the other determiners, the female one for example, can be in two quantities (2*x*) or in single quantity (1*x*). If the two quantities of femaleness overpowers the action of male gene outside the x chromosome, while the single quantity of female genes in single x chromosome, yield to the same group of male genes outside the x, then the sex chromosomes determine sex by controlling the balance between female and male sex determiners or more correctly between their respective actions during sexual differentiation. An improper balance will produce a condition intermediate of the two sexes called intersexuality."

The above discussion indicates that male and female do not differ in the kinds of genes they contain but in their relative amount. It is the interaction of two kinds of genes in different proportion that produces the two sexes. This theory thus satisfies and explains the presence of supersex, intersex and normal condition in nature as well as it accounts for the fact that maleness and femaleness are extremes in the same quantitative series.

6. Hormonal Control of Sex Determination

Several cases of sex determination being controlled either by external environment or by hormones are now known. In *Bonellia viridis*, the sex is controlled by the habitat of larva. The larva which is genetically and cytologically similar, if settles near the proboscis of an adult female, it becomes a male individual and if in free water or on stones, it develops into a female. Likewise, if partly developed male larva is detached from this proboscis of female, it becomes an intersex. However, this example is sometimes referred as sex-determination by environment.

Another case of sex determination being controlled by hormones is that of Crew's hen. Its an example of sex reversal and is named after the discoverer (Crew, 1923). He observed that a female fowl (hen) which earlier laid offspring changed over to fully fertile male (cock). It happened due to damaged ovary in the female and is explained on the basis that ovary in the female secretes a male suppressing hormone under normal conditions. In absence of ovary, testes could develop.

DETAILS OF SOME EXAMPLES OF SEX DETERMINATION

(i) Sex Determination in *Drosophila*

Drosophila has been used as a classical material in the study of sex determination. It has been found that x-chromosome favours

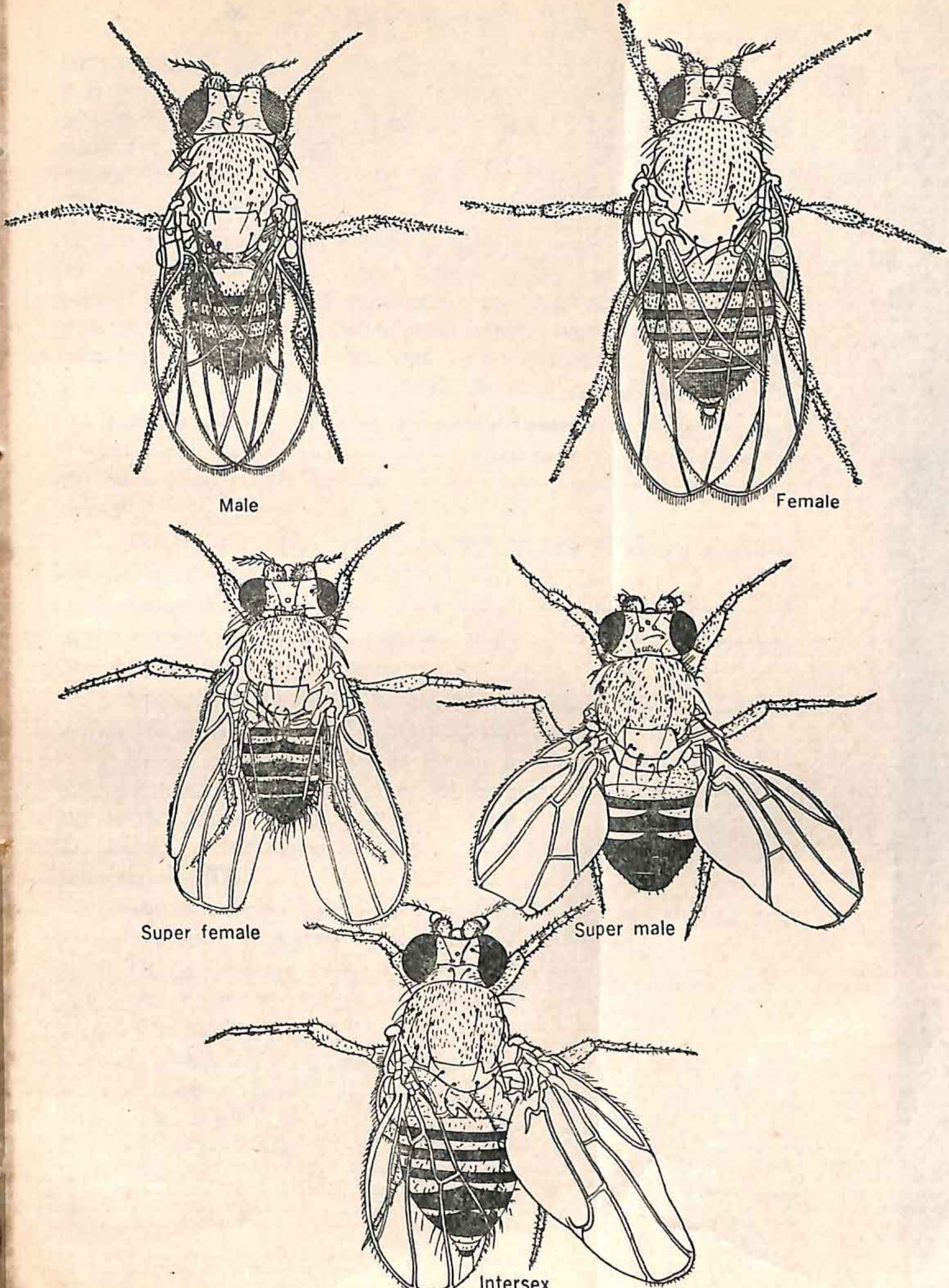


Fig. 13.2 : Different phenotypic forms of *D*

femaleness and the autosomes favour maleness. To this thinking, it is more suggestive that sex determiners are present in both types of chromosomes but male determiners predominate in autosomes while female in *x* chromosomes. It is the balance between *x*/*A* to decide the type of sex to be developed (see "Genic Balance Theory of Sex Determination).

Bridges in his further studies discovered male *Drosophila* with *xo* chromosomal compliment. These males were sterile though normal in behaviour and appearance. He also observed a female with *xxy* composition normal and fertile. The presence of such cases led him to conclude that one *x* chromosome makes a female. *y* chromosome is sexually neutral and therefore inconsequential.

However, on the **role and cytogenetic behavior of *y* chromosome** several sophisticated studies have been made and without affecting the basic concept of the theory following conclusions have been drawn.

1. The *y*-chromosome contains several factors which are essential for the formation of normal sperm.

2. These factors are even active during the diploid phase and due to which *x*-containing sperms (which lacks *y*-chromosome) becomes normally differentiated.

3. The nuclei of sperm have special structures which are observed during the growth phase of primary spermatocytes.

4. These structures develop by an unfolding of *DNA* from chromomeres of *y*-chromosome. It happens in a way similar to the formation of lateral loops in lampbrush chromosomes. In *D. hydei*, 5 loop pairs of different morphology are formed by a relatively small site.

5. The functional nature of these loops have been studied and found that they are related with *DNA*-dependent *RNA* synthesis.

6. The morphology of loops of *y*-chromosome is species specific and the form of loops depends only on factors located in *y*-chromosome.

7. If under any circumstance loop forming site is missing, the male becomes invariably sterile. It has been studied that *DNA* within the loop does not code for protein necessary for the formation of any sperm organelle. However, sperm growth depends on the number of *y*-chromosome present, e.g., males containing two *y*-chromosomes form sperm about twice as long as normal sperm.

Based on these findings Hess and Meyer (1968) propounded a hypothesis to explain the possible physiological singnificance of loops of *x*-chromosome. They stated that "It is assumed that only a relatively short segment of *DNA* in each chromomere is transcribed whereas the rest of *DNA* is not informative, but has some auxiliary functions. These could be to provide a structural substrate for packaging and storing *mRNA* molecules or to accumulate especially large amounts of key substances for spermiogenesis, e.g., specific transfer *RNA*'s which are synthesised during the spermatocyte stage, but used only during spermiogenesis in which stage the nucleus is genetically incomplete and physiologically inactive."

(ii) Sex Determination in Man

In man *xy* mechanism of sex determination is believed to occur. The male potentiality of *y* chromosome is superb as even presence of 3 or 4*x* chromosomes can't change the sex. Besides, one very interesting point about man is the fact that male offsprings are produced in larger number as compared to female. It may be because *y*-chrmoosome in man is only about one half the size of *x*-chromosome, and thereby *y*-chromosome containing sperms are more smaller and active. These sperms have slight advantage in the long race up to reach the upper end of the oviduct where fertilisation is effected. However, problem is still disputable and needs further attention.

Besides, Barr and Betram (1949) observed a significant deeply staining body in the nuclei of nerve cells of female cats and later on in human females also. The structure was named as **Barr body**. Since it was absent in males, it has been considered as sex-indicator. The presence of barr body could be seen in the preparation of skin biopsy, oral epithelium smear, blood smear etc.

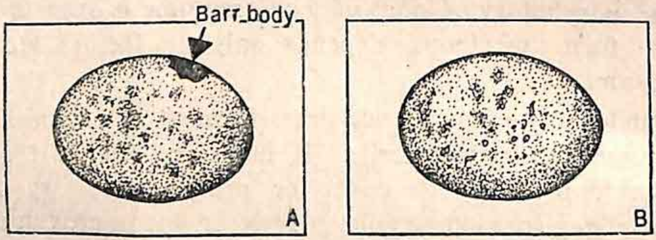


Fig. 13.3. Digram showing the presence of Barr body in the female squamous epithelium (A) and normal male of the same (B).

Davidson and Smith (1954) discovered another difference. They observed a small bulb-like structure attached by a thin thread to the nucleus of some of the polymorphonuclear lymphocytes of female. This accessory nuclear lobule structure was referred as **drum-stick.**

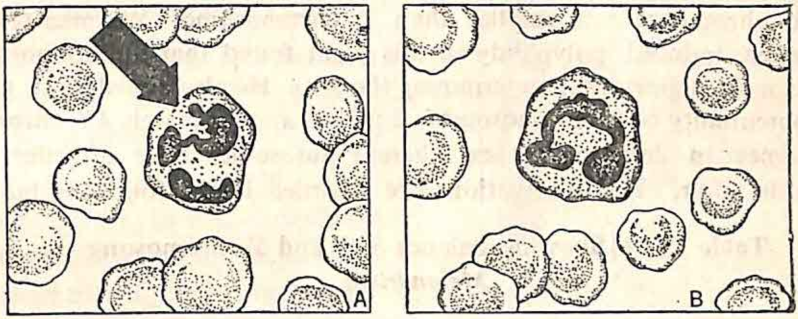


Fig. 13.4: Diagram showing the presence of drum-stick in the white blood cell of normal human female (A) (indicated by thick arrow) and white blood cell of normal human male (B).

Using these structures as sex-indicators, several problems arose when Turner's syndrome and Klinefelter's syndrome were considered. For example, female Turner's syndrome lack barr-bodies while male Klinefelter's syndrome exhibited both barr-bodies and drum-sticks. The problem of these contradictions was resolved by the work of Tjio and Levan (1956). They found that Turner's syndromes were *XO* while male Klinefelter's syndromes that exhibited barrbodies and drum-sticks were *XXY*, *XXXY* or *XXXXY*. All these were sterile.

However, it can be concluded that the role of *Y* chromosome in man is different from that of *Drosophila*. In man, *Y* chromosome is absolutely male determining. Individuals with *XX*, *XXX*, or *XXXX* are, in the absence of a *Y*-chromosome, always female. Although, during recent days certain cases of *XX* individuals with male phenotype while *XY* individuals with female phenotype have been found. It has not been possible to determine whether such abnormal sexual characters are due to hormonal imbalance, chromosomal aberration, developmental disturbance of unknown etiology or the action of individual genes.

(iii) Sex Determination in Higher Plants

(a) MELANDRIUM : It is a dioecious plant belonging to family Caryophyllaceae where sex is determined by a pair of XY chromosomes just as in many animals. It contains 11 pairs of autosomes and XY chromosomes. Morphologically X and Y chromosomes are quite different and pistillate plants are XX and staminate XY. Here X chromosome is smaller than Y chromosome. Warmke (1949) when induced polyploidy in this plant found that Y chromosome is most important in determining the sex. He also noted that male potentiality of Y chromosome is equal to approximately $4X$ chromosomes in determining sex whereas autosomes have no effect in this affair. His observations are recorded in the following tables.

Table 13. II Showing balance of X and Y chromosomes in *Melandrium*

No. of autosomes	*XY complement*	*Sex of plant*
$2A$	XX	Female plant : Normal pistillate and no anthers
$2A$	XY	Male plant : Normal staminate plant,
$2A$	XXY	Male plant : Occasional hermaphrodite blossom
$3A$	XXY	Male plant : ,, ,, ,,
$4A$	XXY	Male plant : ,, ,, ,,
$4A$	XY	Male plant : Staminate
$4A$	$XXXY$	Male plant : Occasional hermaphrodite, blossom
$4A$	$XXXXY$	Hermaphrodite plant : Occasional staminate blossom

Table 13.III. Showing lack of relationship between autosomes and X chromosomes in *Melandrium.*

Autosomes	*X-chromosome constitution*	*X/A ratio*	*Sex*
$4A$	XX	0·5	Pistillate
$3A$	XX	0·67	,,
$4A$	XXX	0·75	,,
$2A$	XX	1·0	,,
$3A$	XXX	1·0	,,
$4A$	$XXXX$	1·0	,,
$4A$	$XXXXX$	1·25	,,
$2A$	XXX	1·5	,,

The detailed study of *X* and *Y* chromosomes of *Melandrium* have been made. *Y*-chromosome is longer than *X*-chromosome. It has been possible to divide these into 5 different segments—(i) segment 1 is suppressing femaleness (*FS*), (ii) segment II promotes maleness, i.e., initiates anther development (*MP*), (iii) segment II controls fertility or late stage of anther development (*MF*) (iv) segment (iv or *PR*) is common in both *X* and *Y* chromosomes and it helps in pairing and regular disjunction of *X* and *Y* chromosome during meiosis and (v) segment (*DR*) is the differential region of the *X* chromosome, which should promote femaleness in the absence of female suppressing segment (i) on *Y*-chromosome.

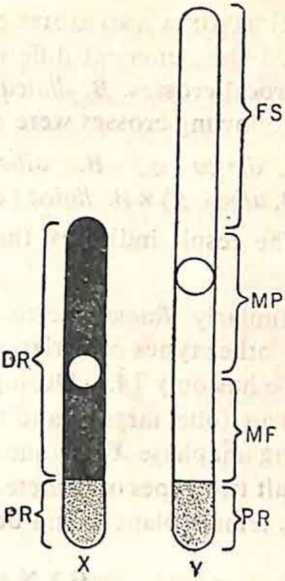


Fig. 13.5. Diagram showing various segments of *X* and *Y* chromosomes of *Melandrium* (*FS*=female suppressor gene; *MP*=male promoter gane; *MF*=male fertility region; *PR*=pairing region and *DR* =Differential region of *X* chromosome).

(b) ZEA MAYS : Maize plant is monoecious but produces unisexual flowers. Male flowers are produced in a tassel at the top of the plant while female inflorescence is axillary. Recent studies have revealed that sex determination in maize is influenced by differential action of single gene and a substitution of 2 single gene pairs makes the difference between monoecious and dioecious plants. The gene for barren plants is referred as *ba* while for tassel seed *ts*. When *ba* (barren plant) is homozygous, it makes the stalk staminate by eliminating the silks and ears and if *ts* (tassel seed) is homozygous, it transforms the tassel into pistillate structure that produces no pollen. Further studies revealed that genotype *ba ba ts ts* lacks silk on stalk but produces only a pistillate plant while plant with *ba ba* ts^+ts^+ is only staminate (male). These data suggest how monoecious plant could become dioecious and *vice versa* by the alteration (or mutation) of two genes.

(c) BRYONIA AND RUMEX : Correns was the first person who demonstrated the genetical differences in the genus *Bryonia* by managing reciprocal crosses. *B. dioica* is dioecious while *B. alba* is monoeciuos. The following crosses were made :

B. dioica (♀) × *B. alba* (♀) = offspring all female plants
B. alba (♀) × *B. dioica* (♂) = offspring 50% male and 50 % female.

The result indicates that male *B. dioica* is heterozygous for sex.

Similarly *Rumex acetosa*, *R. acetocella* and some other species show other types of variation. The male has 15 chromosomes while female has only 14. During pairing at meiosis 6 bivalents and one trivalent (one large *X* and two smaller *Y* chromosomes) are formed. During anaphase *X* chromosome disjoins from *Y* chromosomes. As a result two types of gametes—first with $6+x$ and other with $6+Y_1Y_2$. Thus, female plant wòuld be $12+xx$ and male $12+Y_1\ Y_2$.

GYNANDROMORPHS

The word Gynandromorph consists of three Greek words (*gyne* = woman, *ander* = man and *morphe* = form) and the organism, of which one part of the body has female and other part has male features, is called gynander. Such examples are now and then seen in *Drosophila*, silk moth and beeś. In man, so far only four gynandromorphs are known but only one is survived. Many studies are being made on this surviving human gynandromorph.

KINDS OF GYNANDERS : Three types of gynanders are recognised on the basis of location of sex tissues.

(i) *Bilateral gynanders* : Such organism has half of the body showing female characters while rest half male characters.

(ii) *Anteroposterior gynanders* : Such organisms show anterior region of the body with one kind of sex while posterior half with other sex.

(iii) *Sex piebalds* : In such organism, the female and male sex features are found scattered as spots.

ORIGIN OF GYNANDERS : It has been variously explained.

(*A*) *By Morgan in Drosophila* : In *Drosophila*, the gynander begins to develop with 2*X* chromosomes but one *X* is subsequently lost during cell division. Because of it, half of the body would develop from the cell with 2*X* chromosomes showing female features and rest half with one *X* chromosome showing male features. The gynander can externally be recognised as female portion of body has short wing and abdomen with 5 bands while male by the possession of long wing and 2 or 3 dark abdomen bands as shown in fig. 13.6.

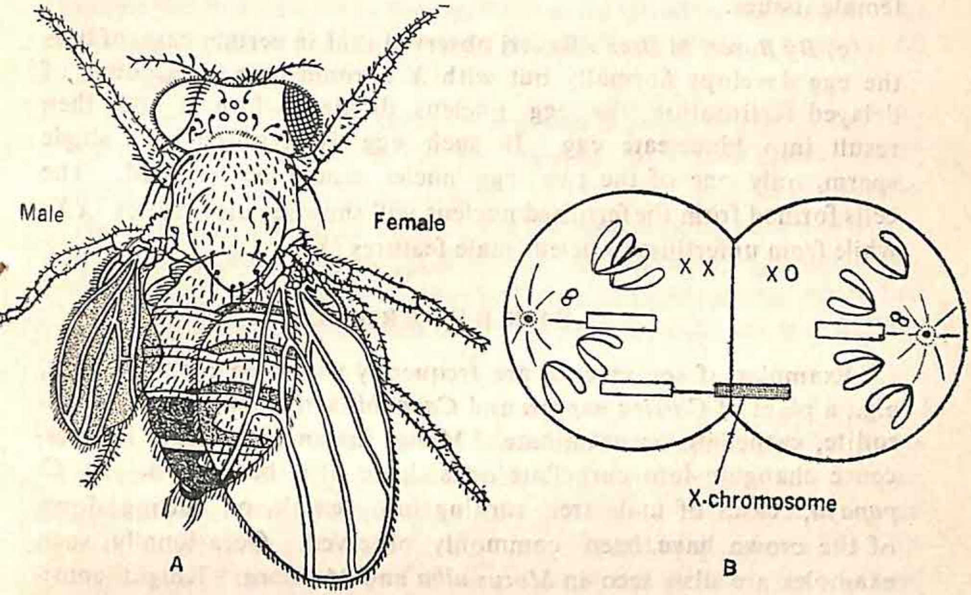


Fig. 13.6 : *A*=*a* gynandromorph of *Drosophila* showing left half male and right half female parts and *B*=the loss of *X* chromosome during mitosis resulting from $2A+XX$ into $2A+XX$and $2A+X$(daughter cells). It explains one of the possible causes of gynandromorphism. The *X*-chromosome labelled below and dotted will be lost during cell division.

In *Drosophila*, gynander may also be formed by non-disjunction of an *X*-chromosome. As a result some cells are *XO* type and others *XXX*. An individual therefore develops part of the body showing male features and other part showing female features. Besides, loss of only a segment of *X*-chromosome (not complete) may also give rise to a gynander.

(B) By Goldschmidt & Catssuki in Silkmoth : In silkmoth, normal female is *XY*. In a normal cell division *X* and *Y* go in separate gametes. Occasionally during cell division, the polar body nucleus remains in

the egg along with the egg nucleus. As a result egg now has two nuclei one with an X and other with Y. During fertilisation, two sperm cells might enter the egg, each one fertilising an egg nucleus. One fertilised nucleus would therfore be XX from which male tissue will develop and other nucleus with XY complement would develop female tissues.

(c) *By Boveri in Bees* : Boveri observed that in certain cases of bees the egg develops normally but with X chromosome on account of delayed fertilisation, the egg nucleus divides before it and then result into binucleate egg. If such egg is fertilised by a single sperm, only one of the two egg nuclei would be fertilised. The cells formed from the fertilised nucleus will show female features (XX) while from unfertilised nucleus male features (X).

SEX REVERSAL

Examples of sex reversal are frequently witnessed among plants, e.g., a plant of *Carrica papaya* and *Cannabis sativa* may be hermaphrodite, carpellate or staminate. Many instances of male inflorescence changing into carpellate ones have also been seen. In *C. papaya*, cases of male tree turning into female on cutting down of the crown have been commonly observed. Occasionally such examples are also seen in *Morus alba* and *M. rubra*. Knight considered them as examples of **Sex Reversal** which he defined as "*A change of sexual nature of an individual from male to female or vice versa.*" Studies on sex reversal mainly based on *Carrica papaya, Cannabis sativa, Lychnis alba, Humulus* sp., *Cucurbita* sp. *Zea mays, Coccinia indica* etc., indicate that it is governed by several factors. Some of them are discussed here.

1. GENETICAL : While working on *Zea mays*, a monoecious plant, Jones noted that gene *Ts ts* pair present on chromosome 1 affects the expression of sex chromosome. He observed in a mutant plant tha trecessive gene '*sk*' in homozygous (*sk sk*) suppresses the development of silk or cob and as a result plant is male. Another recessive gene '*ts*' induces tassel formation and thus restores fertility in female flowers. So female plant is *sk sk ts ts*. Gene '*sk*' has no action in the presence of *ts*. Normal plant is "*Sk Sk Ts ts*" with cobs and tassels.

Jones further noted that in heterozygous condition *sk sk Ts ts* is male which when back crossed (with *sk sk ts ts*) female *sk sk ts* *ts*' and male *sk sk Ts ts* were produced in equal number. The results led him to conclude that *Ts ts* behaves as *xx* and *xy* types.

Hofmeyer (1930) noted that certain genes present on chromosome 2 also behave similarly and affect the sex in maize. He also noted that in *Carrica papaya*, genes M_1 (maleness) and *m* (femaleness) correspond to *xx* and *xy* type as M_1m is male and *mm* is female. The hermaphroditism is determined by yet another gene M_2, i.e., M_2m. All the three are allelomorphic.

In grapes, Valleau observed that sex inheritance is Mendelian and that suppressor of the factor for maleness and femaleness would account for the differing degrees of sexuality and in *Pennisetum typhoides*, Menon observed that the expression of dominant gene for female sterility becomes hypostatic to the plasmon, i.e., particular cytoplasm. Terao (1921) observed that in *Oryza sativa*- sex linked lethal genes cause the death of female gametes and thus semisterility is assumed.

2. CYTOLOGICAL : There have been several cytological causes altering the sex expression in plants. In *Melandrium*, hermaphrodite plants have been obtained by fragmentation of *Y*-chromosome and in *Coccinia indica* normal female ($22+XX$) was highly sterile. Kumar and Vishveswarahiah stated that in *C. indica*, the male organs have developed in spite of the absence of *Y*-chromosome but at the same time there is something in *Y*-chromosome which is necessary for the production of gametes which is absent in the *X*-chromosomes and autosomes. *Cannabis* is another example where various degree of intersexes such as carpellate plants with anthers and male plants with carpels have been seen. It was found that in certain cases pollens had only *x* chromosomes and the carpels produced on male plant contain egg with *x* and *Y* chromosomes. In this case (i) carpellate plants with anthers, (ii) male plants with carpels, (iii) fairly perfect carpellate flowers growing on the upper part of the inflorescence (male intersex) and (iv) very incomplete hermaphrodite flowers may be observed. It is quite interesting to record the observations of Mc Phee (1926) and Hirata (1927,31) that all offspring from a selfed and intercrossed female intersex gave pure female and female intersexes.

Hirata concluded the cytological causes of sex reversal that "the valency of the factors in sex chromosomes is more or less different in practically all individuals so that by crossing there must occur various changes in the relative balance between the action of the two opposing sets of factors. This is in agreement with the fact that intersexes occur when the two opposing valencies tend to cancel each other and the difference between them becomes very small. The nearer the opposing valencies are to equilibrium the more easily is intersexuality produced."

Shifriss (1956) worked on *Riccinus communis* (strictly monoecious plant) and observed that gene *F* controls it. In sex reversals, an unstable nuclear factor brings about the suppression of *F* thereby delaying gene impact, inhibiting male potentiality and stability. The time of sex reversal is under nuclear control. However, nothing is known at present about the nature and location of the unstable nuclear material involved.

3. ENVIRONMENTAL CONDITIONS : Several factors affecting the sex potentiality are known. Among them light, nutrition, abnormal conditions of growth, mutilations, temperature, disease etc. have been reported to induce sex-reversal. In *Mercurialis annua*, *Carica papaya*, *Cannabis sativa*, pruning is commonly known to induce sex-reversals. Sex expression is affected by fumigation with tobacco smoke, photoperiods, abundant moisture, liberal spacing, deeper soil etc. in *C. sativa*. In *Arisoema triphyllum* Schaffner observed that unfavourable dry growing conditions produced maleness while rich wet conditions caused femaleness. In certain cases such as tomato plant, during carbohydrate deficiency development of male organ is suppressed while nitrate deficiency suppresses the female organ. Many cases of this nature are now known.

Similarly hormones also affect the sex expression and result into reversal of sex. Laibech and Kribben (1953) reported that the auxin treatments in *Cucumis sativus* decreased the number of male flowers and increased the number of female flowers. Nitsch (1952) and his co-workers called attention to the profound influence exerted by day length and temperature in sex expression of *Cucurbita pepo* where high temperature and long days tend to keep the plants in male phase.

Naylor and Davies (1950) and Moore (1950) used successfully maleic hydrazide to induce male sterility in maize. Such chemical treatments could substitute for laborious detasseling in the production of hybrid corn seed. Mohan Ram and Chatterjee (1969) collected the gametophytes of *Equisetum ramosissimum* from Yamuna river and concluded that the spores are potential bisexual and the environmental condition determines the sex expression.

GENERAL QUESTIONS

1. Write concise account of sex determination.
2. Discuss critically the chromosome theory and the genic balance theory of sex determination.
3. Write an essay on sex reversals.
4. With the help of suitable examples, explain the sex-determination in plants.
5. Write short notes on—(i) Gynandromorphs, (ii) *Y*-chromosomes in *Drosophila*, (iii) Sex determination in human beings, (iv) Role of *Drosophila* triploids in sex determination.

■ ■ ■

CHAPTER 14

SEX-LINKED INHERITANCE

1. Sex-linked Inheritance in *Drosophila*

(a) RED-EYED FEMALE × WHITE-EYED MALE : While working on *Drosophila melanogaster*, Morgan (1910) found one white-eyed male from a stock of typically red eyed flies. He made a careful analysis of such mutation and got advantage of it. He mated this white-eyed male to a normal red-eyed female and obtained F_1 hybrid which were all red-eyed. When the females of F_1 hybrid were crossed again with normal red-eyed males, half of the males produced were white-eyed and half red-eyed and all females were red-eyed. He further observed in subsequent experiments that half of the females were white-eyed and half red-eyed. These findings led Morgan to postulate the hypothesis of sex-linked inheritance. He explained his findings in a simple way assuming that the gene of the sex-linked character located on X-chromosome (sex chromosome) of the mutant male. During gametogenesis two types of gametes are produced— one carrying the X-chromosome with the white-eyed gene and the other with Y chromosome which on fertilisation with normal red-eyed female gametes will form two types of combination —XX and XY. The XX combination will contain the mutated sex chromosome (i.e., one X from male) and will develop into a red-eyed female F_1 hybrid, the red-eye being dominant over white eye. Now F_1 female will produce two kinds of gametes in equal number—one with normal X-chromosome and other containing white-eyed gene carrying X-chromosome. When these are mated with normal red-eyed male, which also produces two types of gametes one with X-chromosome and other with Y-chromosome, all the female produced will be red-eyed while 50% maie offspring will be white-eyed and rest 50% male with red eyes (Fig. 14.1). It happens so because F_1 hybrid female possesses one X-chromosome normal and another X-chromosome carrying gene for white eyes. Thus, he established an amazing fact that the gene for white eye is linked with sex-chromosome.

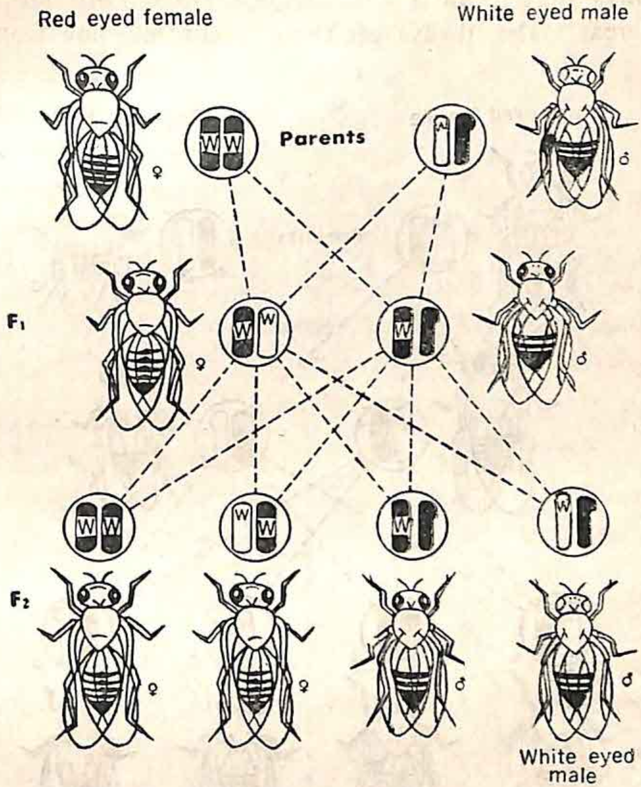


Fig. 14.1 : Diagram showing inheritance of white eye, a sex linked character, in *Drosophila*. A cross is made between a red-eyed female and white eyed male. F_2 shows all red-eyed female and males are red and white eyed in 1 : 1 ratio.

(b) WHITE-EYED FEMALE × RED-EYED MALE : It may also be referred as **reciprocal cross** and **criss-cross inheritance.** Morgan obtained white-eyed female on repeated crossing between F_1 hybrid red-eyed female (which carried one X-chromosome possessing gene for white eye) and white-eyed male. This cross gave 50% female red-eyed and 50% white-eyed female. After obtaining white eyed female he managed a reciprocal cross, i.e., a cross between white-eyed female and red-eyed male. Since mother was white-eyed, it carried both the X-chromosomes possessing genes for white eyes. In F_2, both 50% male as well as 50% females were white-eyed.

In F_1 hybrids, females were red-eyed and males white-eyed because female always inherit an *X*-chromosome from both father and mother whereas males always get their *X*-chromosome from their mother.

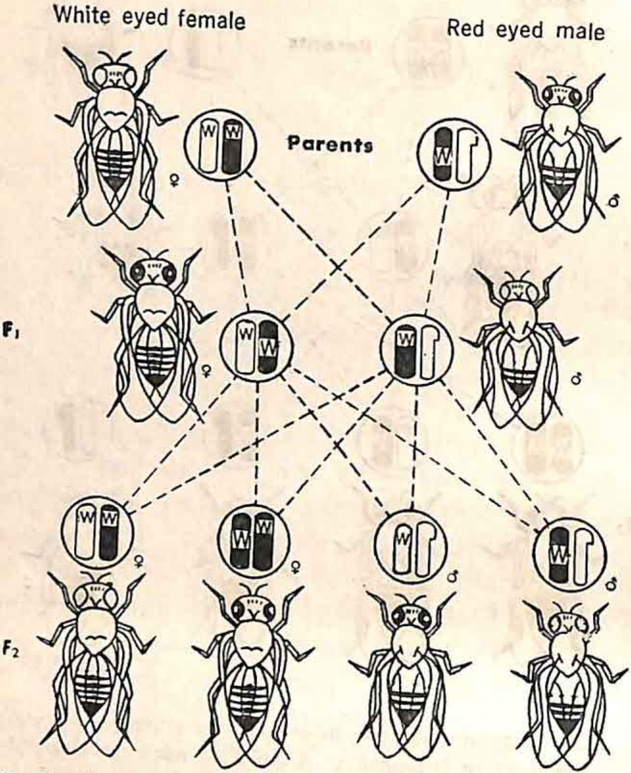


Fig. 14.2 : Sex-linked inheritance of white eye colour in *Drosophila*. When the cross is made between white-eyed female and red-eyed male, in F_1—female offsprings are red and male are white-eyed and in F_2—red and white eyed males and females are obtained in equal proportion.

Thus, in the above crosses, the male transmits his sex-linked traits to his grandson through his daughter and never through his sons and the sex-linked trait appears to alternate or cross from one sex to other in its passage from generation to generation. This phenomenon is called **criss-cross inheritance** and also called to some extent **Nasse's law**. Nearly 140 sex-linked genes are now known in *Drosophila*.

Sex-linked Inheritance in Man

(a) COLOURBLINDNESS—The colourblind man fails to differentiate between the various shades of the red and the green colours. It may also be called as red-green colourblindness. Red blindness is individually called **protanopia** and green blindness **deuteranopia.** The colourblindness is hereditary and the genes responsible for them are sex-linked and recessive to their normal alleles.

The colourblindness is common to male and rare among females. It is because of the fact that colourblindness is a recessive character and its gene is present in *X*-chromosome. In male *X*-chromosome is single and therefore, the trait is expressed while in female the dominant gene present in its another *X*-chromosome is also present that suppresses the expression of colourblindness. The fig. 14.3

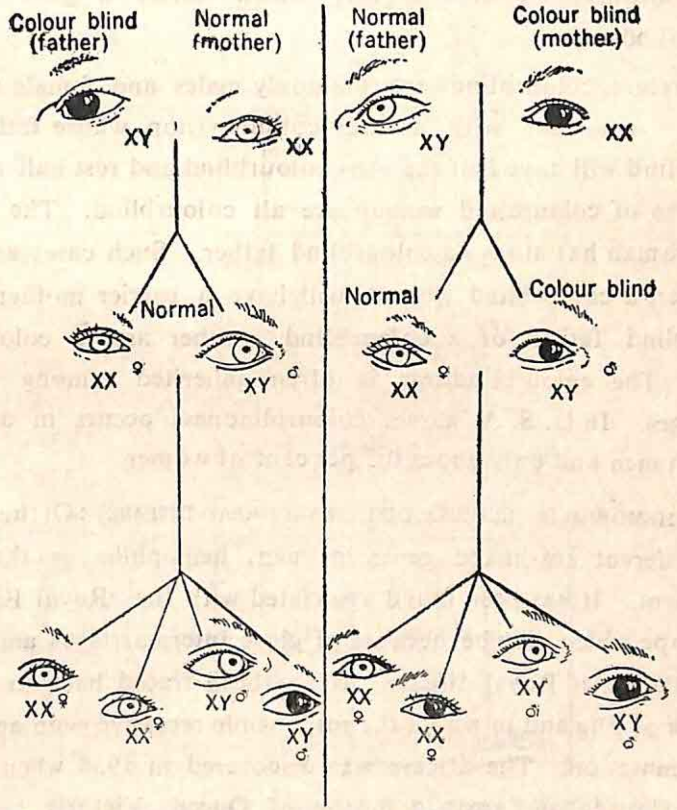


Fig. 14.3 : Sex-linked inheritance of colourblindness in man. The left part of the diagram shows the results of a cross made between a normal woman (mother) and colourblind man (father) while right part of the diagram shows the result of reverse cross, i.e., cross between a colourblind woman and normal father.

gives a clean picture of the transmission of a sex-linked inheritance of colourblindness.

The mechanism of transfer of gene for colourblindness is simple and can be further explained if *C* is taken as a symbol for normal gene and *c* for colourblindness on the *X*-chromosome (criss-cross inheritance). When a colourblind woman (*cc*) is mated with a normal man having *C* at *X*-chromosomes, all the daughters will have normal vision but all the sons will be colourblind because daughters will receive the dominant gene *C* from father while sons will receive the *X*-chromosome from mother which carries a gene (*c*) for colourblindness.

In nature, colourblinds are commonly males and female acts as carrier. A woman with normal colour vision whose father was colourblind will have half the sons colourblind and rest half normal. The sons of colourblind woman are all colourblind. The colourblind woman has always a colourblind father. Such cases are rare. However, a colourblind woman will have a carrier mother and a colourblind father or a colourblind mother and a colourblind father. The colourblindness is often inherited among cousin marriages. In U. S. A. alone, colourblindness occurs in about 8 per cent men and only about 0·5 per cent of women.

(b) HEMOPHILIA (BLEEDER DISEASE OR DREAD DISEASE) : Of the known fifty different sex-linked genes in man, hemophilia is the most prominent. It has been found associated with the 'Royal Families' of Europe which may be because of close intermarriages and that's why often called Royal disease. It has been traced back to Queen Victoria of England in whom the responsible recessive gene appeared due to mutation. The disease was discovered in 1938 when Prince Count Covadonga, great grandson of Queen Victoria, received minor injuries on his face in a motor accident in America and as a result of which he bled to death. In hemophilia, the blood does not clot normally or process is so slow as to cause death due to excessive bleeding. When studied the pedigree record of her family, surprisingly it was found alternating from one sex to other in successive generations and appeared only in men which transmit the disease to their grandsons through their daughters.

The mystery could be explained when it was considered associated with X-chromosome. Hemophilia is a recessive character

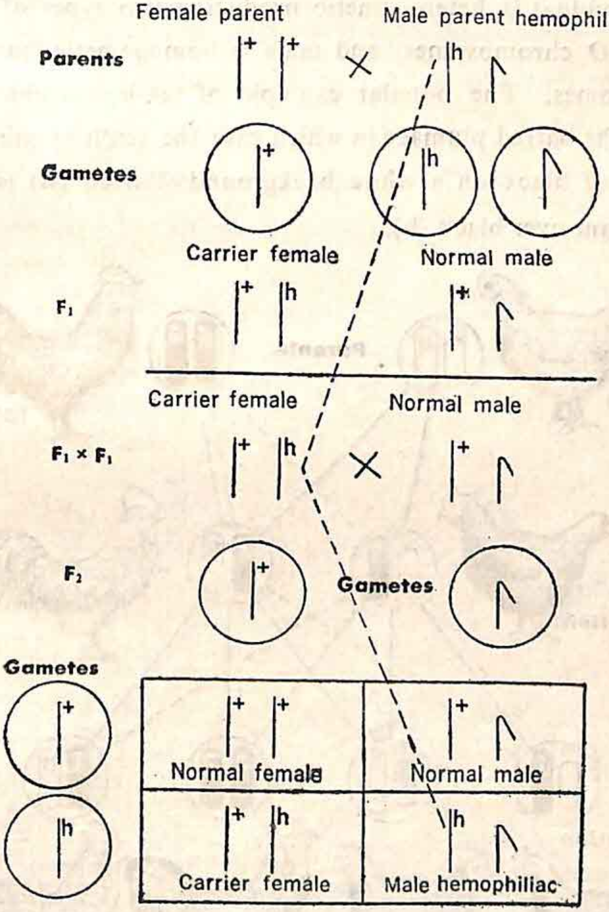


Fig. 14.4 : Diagram showing a cross between a normal woman (female parent) and hemophiliac man (hemophiliac father). The dotted line illustrates the criss-cross pattern of inheritance, i.e., from father through daughter to grandson. The symbol h is to represent the sex-linked recessive gene for hemophilia.

and is, therefore, masked in the heterozygous condition. That's why a lady may carry the disease which would be transfered to her 50% of sons even when father is normal.

3. Sex-linked Genes in Poultry

Sex related inheritance pattern in poultry is reverse type where female individual is heterogametic producing two types of gametes (with *X* or *O* chromosomes) and male is homogametic having two *X*-chromosomes. The popular example of sex-linked character in poultry is the barred plumage in which case the feathers are banded with bars of black on a white background. Barred (B) is normal and dominant over black (b).

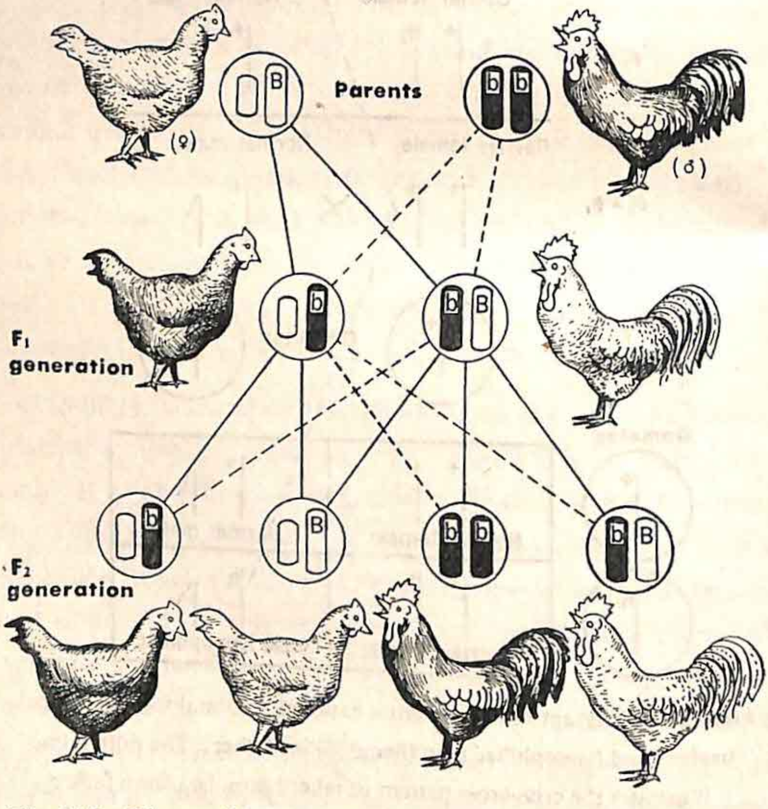


Fig. 14.5 : Diagram illustrating the sex-linked inheritance in poultry. The cross is made between a barred female and unbarred male.

When the cross is made between barred female (B–) and black male (bb) only barred males and black females are obtained in F_1 generation and in F_2 barred and black individuals appear in 1 : 1 ratio among male and female offsprings.

And when cross is made between black female (b–) is crossed with barred male (BB), all the progenies obtained in F_1 would be barred and in F_2, barred and black individuals would be found in 1 : 1 ratio among female population. All F_2 male individuals would be barred.

These reciprocal crosses reveal that a male may carry two genes for barring but a female carries only one.

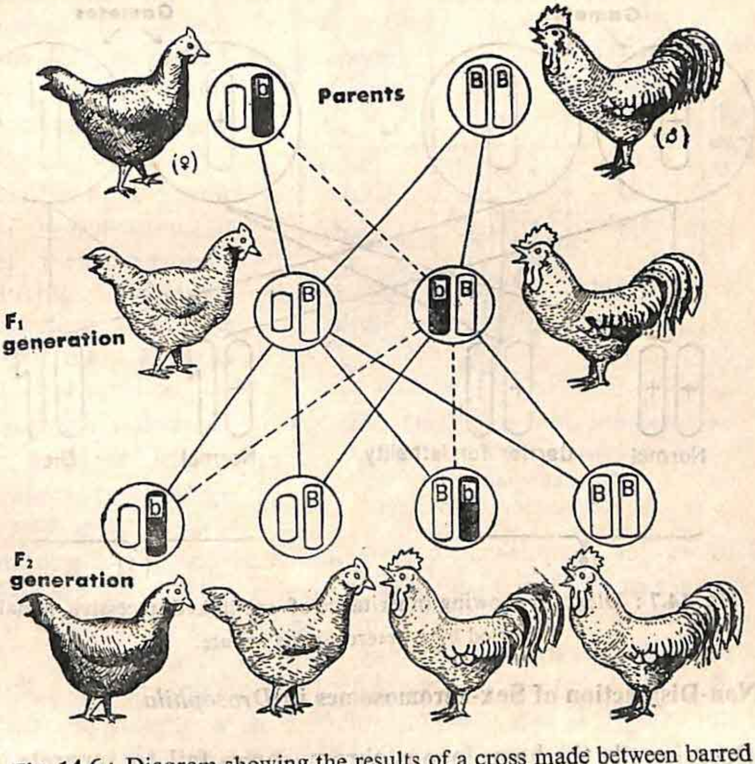


Fig. 14.6 : Diagram showing the results of a cross made between barred male and unbarred female.

4. Sex-linked lethals in *Drosophila*

In certain cases, the females of *Drosophila* regularly produce female and male progenies in 2 : 1 ratio, i.e. to say that half of the male progenies are missing. It happens perhaps due to presence of sex-linked lethal (recessive) in the female parent. Such females are heterozygous. The fig. 14·7 explains the mechanism.

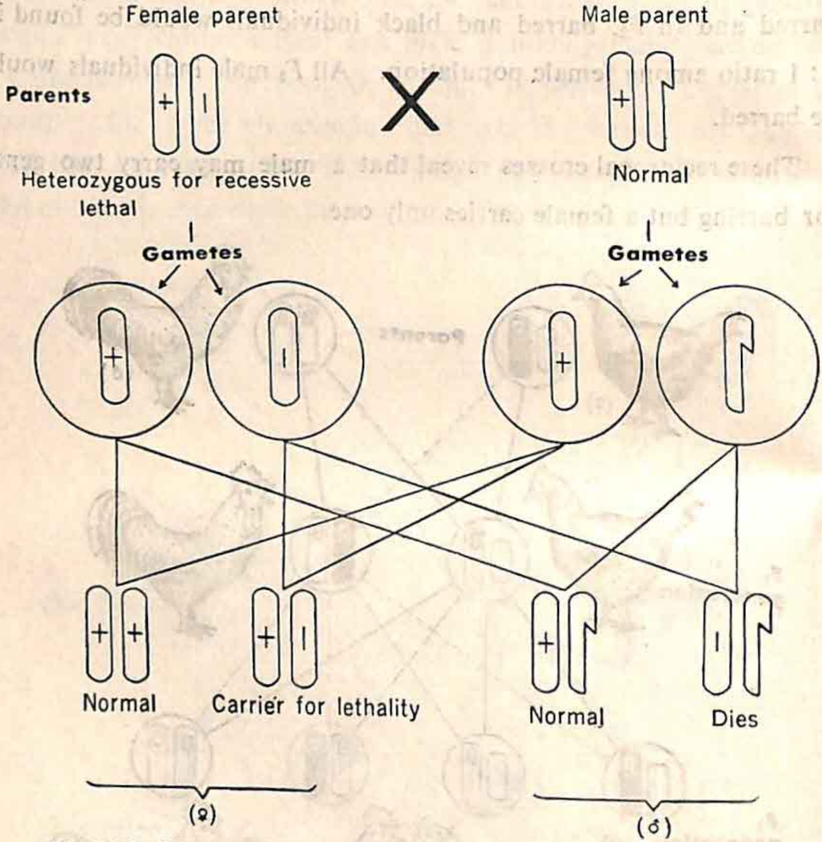


Fig. 14.7 : Diagram showing inheritance of sex-linked recessive lethal carried by a heterozygous female.

Non-Disjunction of Sex-chromosomes in *Drosophila*

Occasionally the homologous chromosomes fail to separate after synapsis during first meiotic division and as a result both chromosomes of the pair goes to one gamete. The other gamete, however, remains deviod of it. It is called non-disjunction.

(a) PRIMARY NON-DISJUNCTION : While working on ***Drosophila***, C. B. Bridges (1916) published a paper on non-disjunction as a proof of the chromosome theory of heredity. He observed that about one fly in 2000 to 3000 F_1 offsprings has the unexpected inheritance of red eye colour in male and white in females. Explaining this fact, he argued that female obtains both its *X*-chromosomes from her mother as a result of non-disjunction of *X*-chromosomes during meiosis. Similarly the red eyed male receives *X*-chromosome from his father.

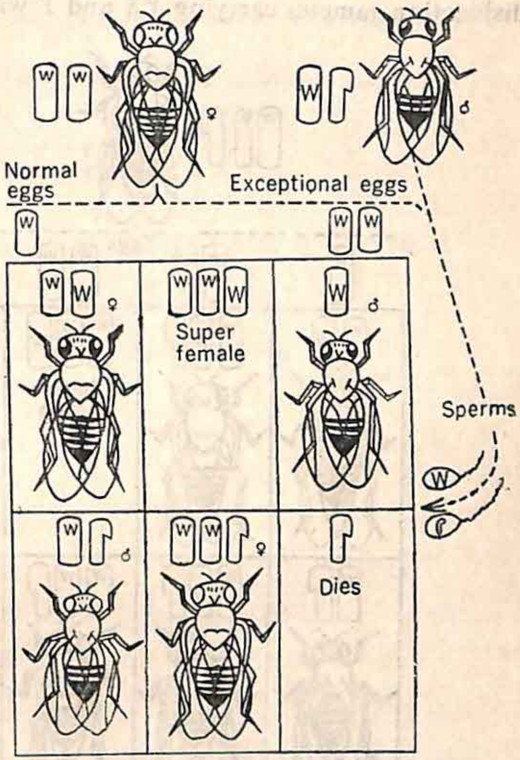


Fig. 14.8 : Diagram showing primary non-disjunction of *X*-chromosome in *Drosophila*.

Bridges further observed cytologically that *X*-chromosomes received in a female may occasionally fail to disjoin at first meiosis. This primary non-disjunction leads to the production of three kinds of eggs as shown in fig. 14.8. When these eggs are fertilised with the male gametes of normal male, four types of offsprings are produced – (i) Red eyed males, (ii) white eyed females, (iii) individuals with 3 *X*-chromosomes and (iv) individuls with no *X*-chromosome. Individuals with no *X*-chromosome dies.

(b) SECONDARY NON-DISJUNCTION : Bridges extended his experiment ahead using *XXY* females in further experiments and obtained cases of secondary non-disjunction. In a cross between *XXY* female and red eyed normal male, he observed red eyed females 96%, and white eyed females only 4%. This led to conclude the occurrence of secondary non-disjunction upto 4%. Due to secondary non-disjunction gametes carrying *XX* and *Y* will be produced.

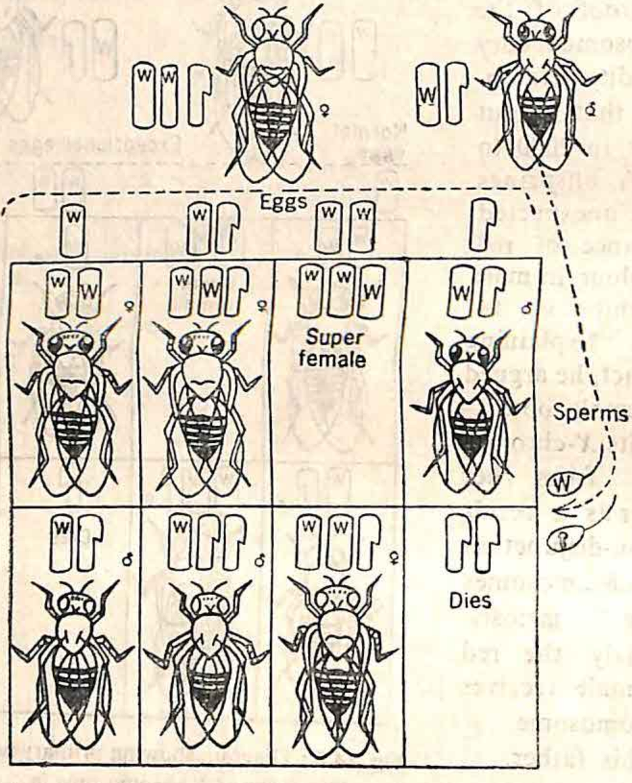


Fig. 14.9 : Diagram showing secondary non-disjunction of *X*-chromosome in *Drosophila*. The cross is being made between white eyed female and red eyed male.

From the white eyed *XXY* females, four types of eggs are produced as shown in fig. 14.9 that on fertilisation with normal red-eyed male would produce eight types of progenies. The results obtained are shown in the figure.

These experimental findings serve as an evidence to show that the genes or Mendelian factors are carried by the chromosomes, chromosome theory of inheritance. The expected presumptions such as occurrence of *XXY* females were cytologically confirmed.

GENERAL QUESTIONS

1. Some diseases are inherited sex-linked. Discuss them with the help of suitable examples.

2. Write short notes—(i) Colourblindness, (ii) Hemophilia, (iii) Primary and secondary non-disjunctions.

■ ■ ■

CHAPTER 15

MUTATIONS

Mutation is considered as one of the causes of variation in the nature but it includes only heritable changes which alters the phenotype of the organisms. While working on *Oenothera lamarckiana* Hugo de Vries (1901), one of the rediscoverers of Mendel's laws, introduced the term 'Mutation' and proposed 'The Mutation Theory' (die Mutation theory). He observed nearly 834 variations in the plant, one of them *gigas*. The gigas form differed from the parental plant in its large size. Later it was found that what de Vries described as mutations were not actually gene mutations. Gigas was only a tetraploid (polyploid) with 28 chromosomes instead of 14 in the parental form.

However, de Vries proposed that these mutations constitute a distinct field in the study of variability. These are without transitional gradations and are rare unlike continuous variations as proposed by Darwin. He further believed that mutations give rise not only to varieties but also to species. Such large mutations were called as **sports**. Mutations, when they occur, may be responsible for the origin of new species which differ both in phenotype and genotype from their ancestors. They have new morphological and genetical characters. Mayr (1942) also defined it as "*Mutation is a discontinuous chromosomal change with a genetic effect.*"

While working on *Drosophila melanogaster* T. H. Morgan in 1909 discovered mutation that was proved to be really due to a gene. He reported the occurrence of white eyed individuals among red eyed individuals due to mutation. Since then several other cases (some 500 cases) of mutation have been discovered. Some of them were large enough to be seen by simple eyes and others were too minute to be detected easily. The work on mutation has also been done in other organisms such as maize, snapdragon, rodents, pea, fowls, man etc. During the recent years, geneticists have developed interest on mutational studies in micro-organisms like *Neurospora*, Bacteria (*Escherichia coli*) and bacteriophages. These materials have been found to be very suitable for mutation work.

During course of researches on mutation, a milestone was laid by H. J. Muller (the student of T. H. Morgan) when in 1927 he discovered that mutations can be artificially induced by X-rays in *D. melanogaster*. His arduous labour in the study earned him in 1946 The Nobel Prize for Physiology and Medicine. Since then several mutagenic agents have been discovered.

Some of the important points related to the nature of mutation are summarised here to outline its brief important aspects.

1. Gene is the unit of mutation and the mutated gene is as stable as the normal gene.
2. Different genes show different rates of mutation.
3. It may occur at any stage of life cycle.
4. The rate of mutation may vary in different tissues or at different stages of development of the organisms.
5. Mutation is not a loss of gene rather a change in gene.
6. More than one change may occur in a gene. It may produce multiple alleles, which, while, usually affecting the same character in different degrees may effect different characters. The multiple allelic mutation of a given gene may not always be in the same direction.
7. The mutation appears to be a chemical process.
8. Mutations are usually injurious.
9. Mutations are usually recessive to the wild types.
10. It does not ordinarily occur in more than one gene at a time.
11. The two members of a pair of gene mutate independently, just as different genes do.
12. Minute mutations are more common than larger ones. Mutations with no visible effects are the most common of all.
13. Mutations are haphazard not only in the nature of their effects but also in points of their origin.

Detection of Mutation

(A) THE *ClB* METHOD : This method involves the use of *ClB* stock of *Drosophila*. One of the two sex-chromosomes in female carries three features, collectively called *ClB* (*C* for an inversion in the heterozygous state to work as cross over suppressor; *l* for a recessive lethal in heterozygous state and *B* for barr eye or narrow eye). To begin with the actual process, the normal male fly is irradiated for the induction of mutation and then they are crossed to *ClB* females. The results of the cross is shown in the fig. 15.1 in which case male

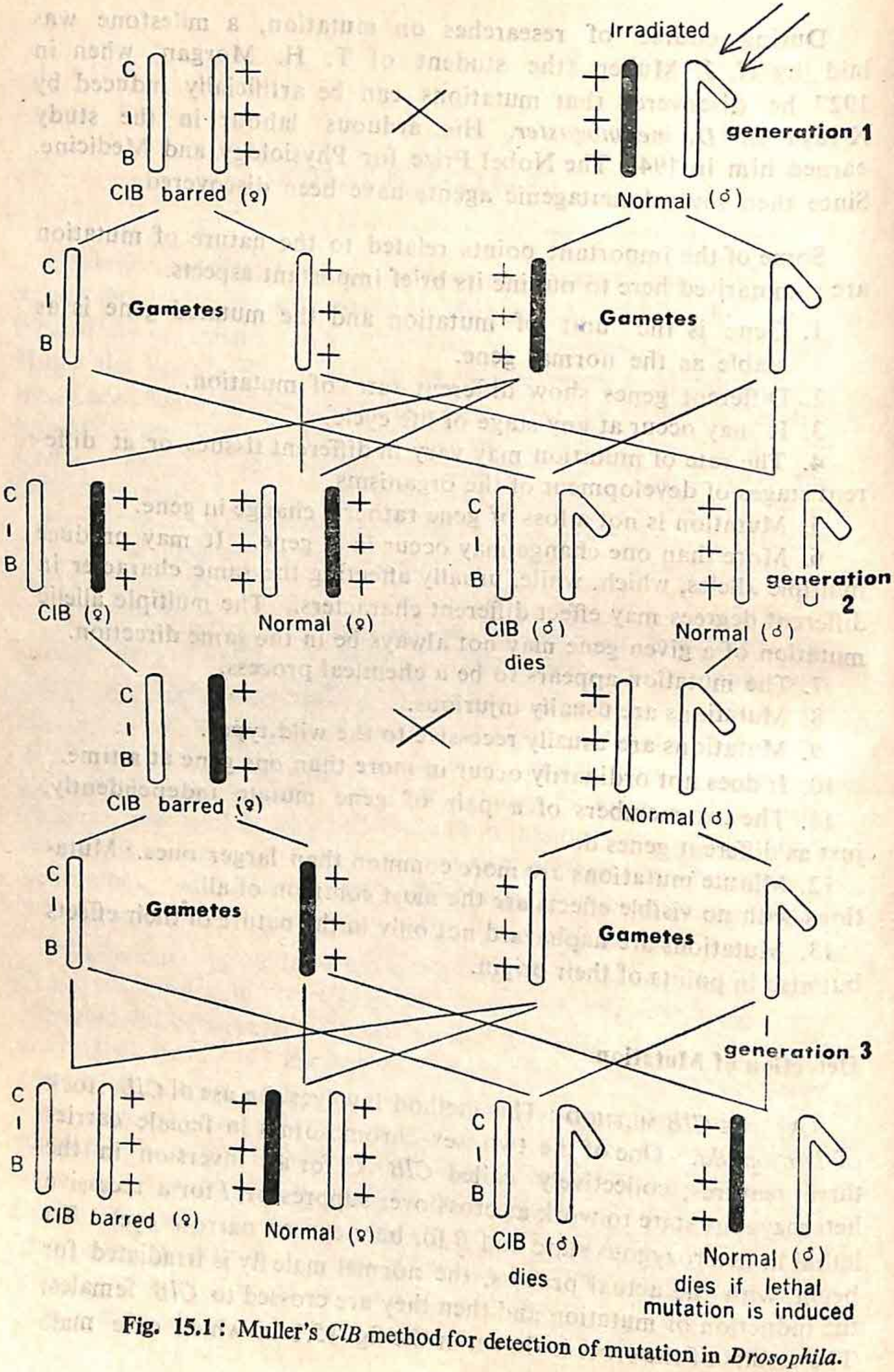


Fig. 15.1 : Muller's *ClB* method for detection of mutation in *Drosophila*.

progeny that receives *ClB* chromosome will die. The *ClB* female are selected by their barred eyes which are crossed to normal males. In the offsprings so produced 50% male will again die. They will be *ClB* males. The remaining 50% males will receive *X*-chromosome which may or may not carry the irradiated induced mutation. If at all in the process lethal mutation has been induced in the male, these male will also die. As a result, no male will be produced. So the absence of male will indicate that mutation has occurred.

This method is also referred as a method for detecting sex-linked lethal mutations.

(B) MULLER'S 5 METHOD : This method is being carried on special Muller-5 *Drosohpila* which carries two marker genes—the dominant **Barr** (**B**) and the recessive **apricot** (**apr**). The apricot gene is not as lethal as in *ClB*. The method is similar to that of *ClB*. In the generation 2, 50% males are Muller's 5 type and the remaining 50% are wild type. It is to be seen that if mutation in *X*-chromosome of irradiated male is induced, there would be no wild type male in generation 2. The absence of wild type in generation 2 will be therefore an indication of an induced lethal mutation. (Fig. 15.2)

(C) DETECTION OF MUTATION IN PLANTS : Singleton (1962) while studying induced mutation proposed a method of its detection in plants. He selected pollen from the plants which were dominant for several characters and were grown in the field with radiation source. These pollens were used to pollinate plants with recessive characters and grown in the field without radiation source. The seeds obtained from such a cross were grown and observed that many recessive characters appeared. It indicates that mutation has been induced in the plant which changed some of the dominant characters.

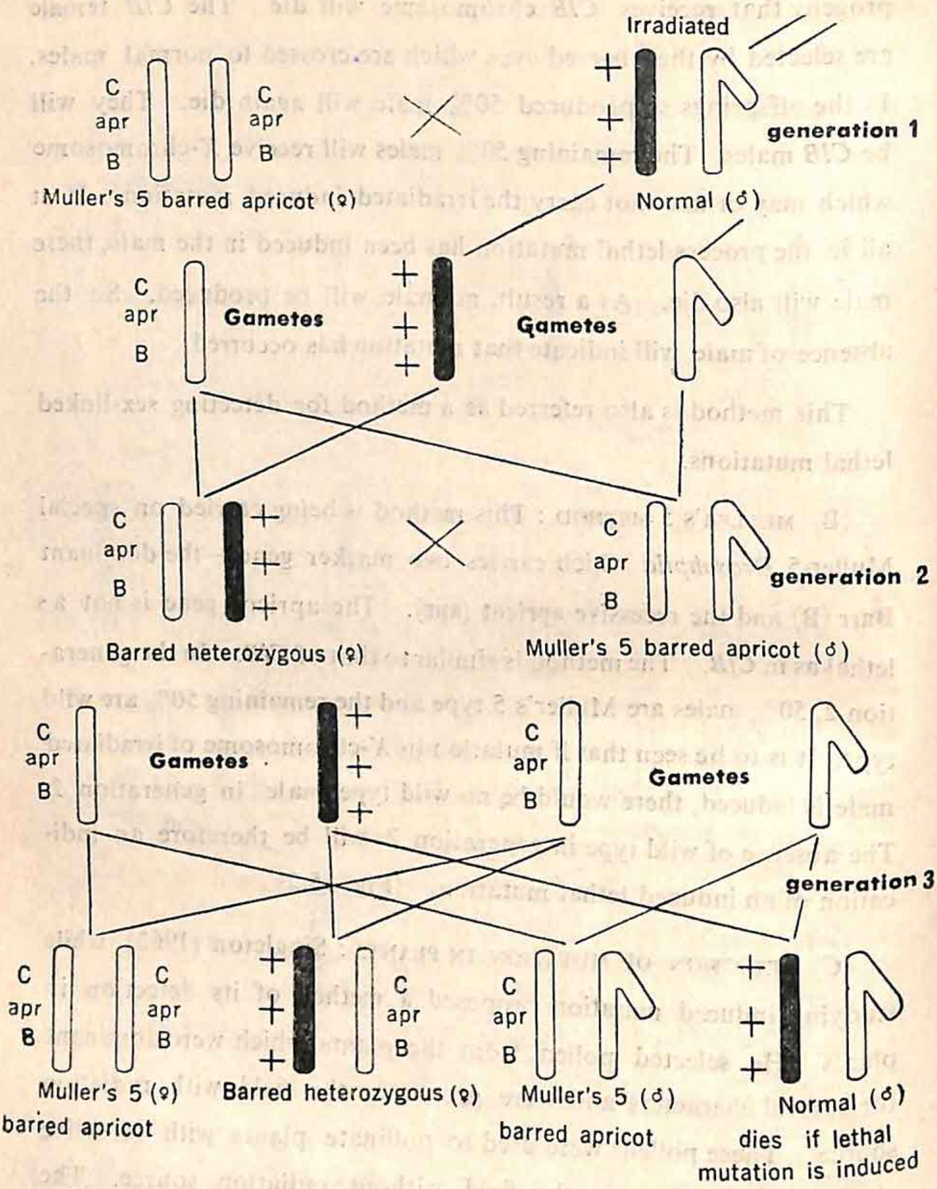


Fig. 15.2 : Diagram showing Muller-5 method for detection of sex-linked mutations in *Drosophila*.

Classification of Mutation

Earlier chromosomal aberrations and polyploidy were also considered within mutation, but in recent interpretation of the term mutation it is referred to the gene or point mutation. The mutation may be further classified on the basis of its type and conditions of its occurrence in the organisms.

1. GERMINAL MUTATION : This kind of mutation is found in the reproductive cells and is heritable.

2. SOMATIC MUTATION : When it is found in the vegetative cell it is called somatic mutation. It is non-heritable.

3. SPONTANEOUS MUTATION : When mutation occurs in nature it is called spontaneous mutation.

4. INDUCED MUTATION : When mutation is artificially produced using irradiations or mutagenic chemicals (various mutagenic agents).

5. REVERSE MUTATION : When mutated gene again mutates to become normal, it is referred as reverse mutation.

6. SPURIOUS MUTATION : It is a kind of hidden mutation in the recessive gene which appears phenotypically by the crossing over as gene for pink eye colour in *Drosophila*.

7. BIOCHEMICAL MUTATIONS : When mutations affect the specific biochemical processes in the individuals, it is called biochemical mutation. Many cases of such mutations are known in *Neurospora*.

Mechanism of Mutation

While considering the mechanism of mutation it should come in the mind that it is a chemical change in the genetic material. Experimental findings have also supported this fact. It is considered as a chemical change because

(i) the end product of the change is always same, no intermediate form.

(ii) change is always not a random process, it may be favoured by certain tissues or may even be limited in certain tissues.

(iii) there has been found various genetic factors which stimulate the rate of change in certain unstable gene.

(iv) the process is also reversible, some times.

Frobisher (1968) has rightly defined mutation as *sudden change inheritable characters that is not a result of transfer, segregation, or recombination of normal genes, but of an alternation of the numbers, molecular structure or sequence of nucleotides that constitute the genes themselves.*

SPONTANEOUS MUTATION : The frequency of spontaneous mutation is extremely low often in the range of 10^{-8} to 10^{-10} for each replication of genome. In case of spontaneous mutation, the recent evidences indicate that chemical change within the cell can provide the necessary energy through chemical metabolites that assume the role of mutagens.

While discussing the mystery of spontaneous mutation, Serra (1968) reported that polypeptide chains provide a means for the propogation of excitation of energy. In one analysed case the energy was passed in the direction phenylalanine→tyrosine→tryptophan. He stated that it seems very likely that the energy may also be transferred to nucleotides or possibly transmitted along the nucleotide chains themselves. Similarly, Little (1965) has suggested that organic molecules, especially like *DNA*, with suitable arranged side groups may have superconductivity at room temperature.

Watson (1965) while explaining the cause of mutation stated that it occurs mostly during replication as a failure. Either the correct hydrogen bases do not form or adenine mistakingly pairs with guanine. Such highly mutable nucleotides are known as **hot spots.**

INDUCED MUTATION : (A) *Radiations* : Various kinds of irradiations used commonly for mutagenic treatment are known since 1930 or so. Commonest of the use are X-rays and ultra violet light. Although no precise mechanism is proposed to explain the mutagenic effect of **X-rays**, it is believed that they cause the rupture of many different kinds of chemical linkages and thus damage the genetic material in a variety of ways. X-rays and gamma rays are ionising radiations and have high penetrance and, therefore, can induce mutations even in seeds.

Ultra-violet Light is mutagenic but only effective on bacteria, fungal spores and other free cells whose genetic material is not shielded by any great thickness of ultra violet absorbing material. Its mode of action can be explained more clearly. It causes the formation of pyrimidine dimers (i.e., linked pairs) especially the linking of thymine side chains of *DNA* strands.

Such interstrand dimerisation by producing cross links between nucleic acid chains inhibits strand separation and distribution. Thus the formation of thymine dimers has been proved as a major cause of killing of cells by *UV*.

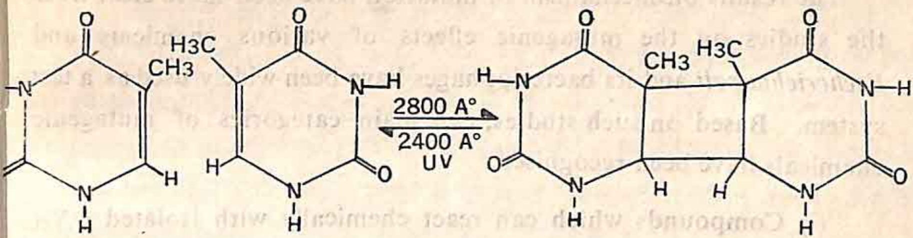


Fig. 15.3 : Diagram to explain the formation of thymine dimer when treated with *UV* 2800 Å. However, normal condition could be restored at *UV* 2400 Å.

The site of mutagenic action of *UV* in T_4 bacteriophages is the guanine-hydroxymethyl cytosine. It brings about deamination of hydroxymethyl cytosine or cytosine to give hydroxymethyluracil or uracil which would be expected to pair with adenine at the following round of *DNA* replication.

Gamma rays, which are of shorter wavelength than X-rays, have been frequently used for the induction of mutation in crop plants during recent years. I.A.R.I., New Delhi maintains a gamma garden in a three acre plot. Radioactive cobalt (Co^{50}) is used as a source of gamma rays. It has been erected in the centre around which plants in pots are kept at varying regular distance which are then irradiated and studied.

(**B**) *Chemical mutagens* : Many chemicals are known to induce mutation. Some of the important chemical mutagens are—Colchicine, Tetramethylene-diamine (amide), Rhodanide (*KCNS*), Allyl-isothiocyonate or mustard oil, Formaldehyde, Citronellal, Morphine, Narceine, Atropene, Scopolamine, Phenols, Quinols, Coumarine and its derivatives, Caffeine, Theophylline, Theobromine, Ethylmethyl sulfonate, Acridine, Diethyl-sulfate, Dimethyl-sulphate, Bromomethyl methane sulfonate, 2-aminopurine, 5-bromouracil, Nitrous acid, Proflavin, Maleic hydrazide, Hydrazine, Diepoxybutane, Dimethyl-nitrosamine, Hydroxylamine, Myleran etc.

The results on mechanism of mutation have been more clear from the studies on the mutagenic effects of various chemicals and *Escherichia coli* and its bacteriophages have been widely used as a test system. Based on such studies, two main categories of mutagenic chemicals have been recognised.

(i) Compounds which can react chemically with isolated *DNA* and can induce mutations in bacteriophages (chemically reacting compounds).

(ii) Base analogs which are sufficiently similar to normal *DNA* bases to substitute for them during replication (substitution of base analogues).

(I) *Chemically Reacting Compounds* : The effective mutagens of first group are the alkylating agents of which mustard gas and its other compounds were investigated earlier. But the use of ethylating and other alkylating agents are now commonly used. A few of them are Diethylsulfate, Ethylethane sulfonate and Ethyl methano-sulfonate. They react with guanine to give 7-ethylguanine which soon destabilises the base deoxyribose linkage. As a result guanine readily splits leaving a gap in *DNA* chain. The *DNA* strand missing a base will incorporate any one of the four bases at random opposite the gap at the next replication. In the second case 7-ethylguanine may not split from *DNA* chain but may bring out mutation by forming a hydrogen pair with thymine instead of cytosine.

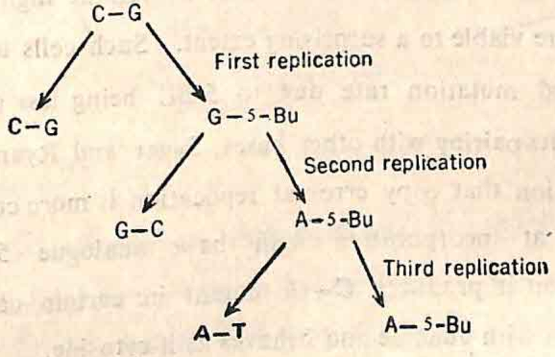
Hydroxylamine acts in a different way. It reacts only with cytosine and not with any other base of *DNA*. **Nitrous acid** is long known for its reaction with amino group ($-NH_2$) of bases and to form hydroxyl group by liberating nitrogen gas. As a result, adenine is converted into hypoxanthine, guanine into xanthine and cytosine into uracil. Following the replication, the *DNA* base sequence changes as uracil pairs with adenine and hypoxanthine prefers to pair with cytosine rather than thymine.

Any chemical reaction with genetic material may change the process of gene action and ultimately resulting a reproducible change in the spatial configuration of the *DNA* molecule. If it is so, any chemical substance will act as mutagen provided it can penetrate the cell at proper place at the proper time and react with one or more functional groups of the genetic material.

(II) *Substitution of base analogues* : The base analogues do not cause mutation in isolated genetic material rather only in living system. 5-Bromouracil (5-BU) and 5-fluorouracil (5-FU) are close analogues of thymine. They have a bromine or a fluorine atom in place of similar sized methyl group, respectively. Similarly 2-aminopurine (2 AP) is close analogue to adenine. The analogue fits almost perfectly in the *DNA* molecule.

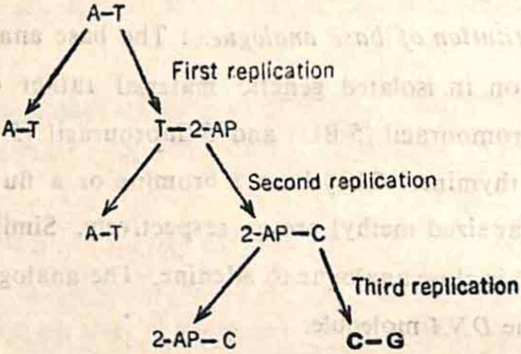
The base analogues cause mutation in two different ways.

(a) *Mutation by mis-incorporation* : 5-BU may be incorporated with guanine in a way as if it is cytosine and then proceed to replicate as if it is thymine. Thus, if 5-BU becomes incorporated opposite to guanine, in the next replication it will behave in the more usual way and will pair with adenine. Ultimately $G-C$ pair will be replaced by $A-T$ pair. It may be explained as follows:



The mutation by mis-incorporation of a base analogue is essentially established at once.

(b) *Mutation by mis-replication* : 2-aminopurine (2AP) correctly incorporates as an adenine analogue and then it replicates at the subsequent divisions in a way as if it were guanine. The entire sequence may be—



The mutation by mis-replication will continue to arise with a constant probability per replication for an indefinite number of cell generations. If in such mutations, adenine is ultimately replaced by guanine (purine base by another purine base), it is called **transition** and if thymine is replaced by guanine (pyrimidine base by purine base), it is called **transversion.**

Fincham (1965) has shown that cells containing high proportion of 5–BU are viable to a surprising extent. Such cells tend to show an increased mutation rate due to 5-BU being less specific than thymine in its pairing with other bases. Sager and Ryan (1963) are of the opinion that copy error at replication is more common than copy error at incorporation with base analogue 5-*BU*. With misreplication it produces *C–G* mutant in certain cases (rarely) when it pairs with guanine and behaves as if cytosine.

In transition category, the mutation due to tautomeric shiftings is another interesting way. It explains that each base of *DNA* molecule can exist in alternative state, e.g., in normal state or in rare state. A rare state of the base occurs by the rearrangement in the distribution of hydrogen atoms in the molecule. It is called **tautomeric shifts.** The rare states of all the four bases are shown here.

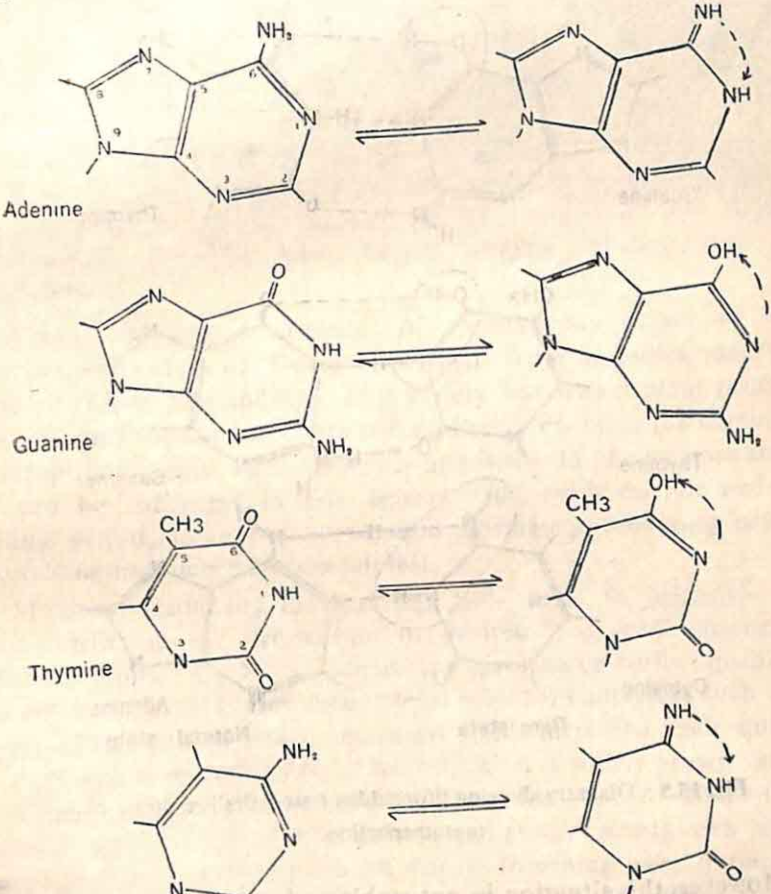


Fig. 15.4 : Diagram showing tautomerism of the bases of *DNA*.

In this concept, if the base is in rare state, it will not pair with its normal partner, e.g., in rare state adenine will link with cytosine rather than thymine. Such pairings are called **forbidden base pairs.**

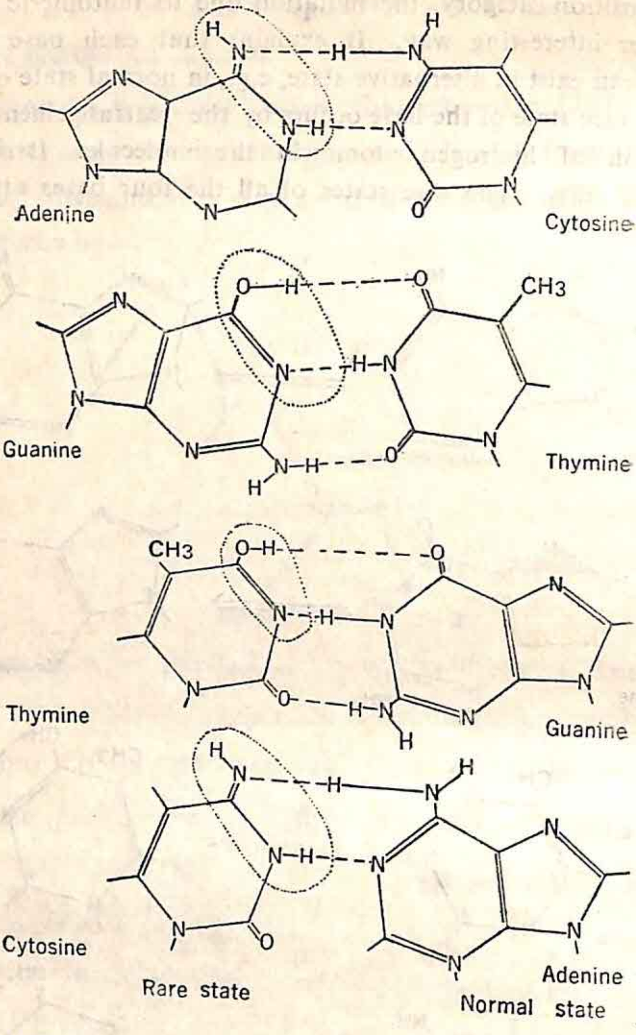


Fig. 15.5 : Diagram showing "forbidden base pairs" resulting from tautomerisation.

However, this situation is not stable and at the next replication, the rare state may change into normal state. But once a forbidden base pair is formed, it will include a changed base. Thus, two kinds of *DNA* molecules—one normal and other that has undergone a base pair substitution (mutated *DNA* molecule) would be formed.

Mutations and Crop Improvement

Several spontaneous mutations proved helpful in the improvement of crops are now known. From rice variety 'konamani' arose GEB. 24, from *Sorghum* Co. 2 arose Co. 19 'Talaivirichan' and from ragi E.C. 593 arose E.C. 3735. A new form 'sweet lupin' arose as a natural mutant from the 'yellow lupin'. Unlike yellow lupin, sweet lupins are relatively free from the high content of alkaloid. In potato, varieties 'Russet Burbank' and 'Red Triumph' arose as natural mutations from varieties 'Smooth Burbank' and 'Triumph.' respectively.

Dwarf wheats are probably the latest type of high yielding wheats. The genes responsible for dwarfness arose as spontaneous mutations in the 'Norin' Japanese wheat. Norin 10 has desired combinations of short plant height, lodging resistance and ear characters.

Among the several instances of spontaneous origin of new varieties, the origin of 'Dee-gee-woo-gen' from an indica variety of Chinese rice is outstanding. This variety has dwarf plant reaching upto 60 cm. and the leaves are stiff and erect enabling the maximum interception of light. The variety is insensitive to photoperiod thereby can be cultivated in any season. The seeds do not undergo resting period, i.e., absence of seed dormancy rendering sowing possible immediately after the harvest.

Mutation artificially induced has been asset to scientists and agriculturists in the production of desired improved varieties of different crops. U. S. A. scientists have successfully produced mutant varieties of barley with several valuable characters such as—increased strength of straw, increased yield, increased malt quality or earliness in maturity etc. 'Bonneville' is a widely grown variety of barley in U. S. A. When formers made complain of difficulty in its threshing, the seeds were irradiated and several plants with brittle awns that were easily broken off during threshing were found. A new variety thus formed was named 'Bonneville 70' which proved a real improvement over earlier one.

A good example of radiation mutation breeding is seen in *Mentha piperita*. From this plant pipermint oil is extracted which is variously used. Its crop was seriously attacked by *verticillum* wilt. To overcome this problem, when attempts to hybridise it with a related species *M. cripsa* and others failed *X*-ray radiation breeding carried out by Murray (1960) became most helpful in producing resistant varieties while still preserving the flavour of pipermint.

Most of the high yielding cultivated varieties are the result of an intelligent and prudent exploitation of natural variations and the altitude among plant breeders toward the possibilities offered by radiation-induced mutations ranges from over enthusiasm to skepticism. Much work has been done on rice and wheat. Such programmes are run either independently or in coordination with number of other countries. Studies have been frequently made on 'japonica' varieties of rice of Taiwan such as Taiwan 3 and Taichung 65 to produce characteristics of the 'indica' type which is not sticky and rich in starch amylose while retaining all other traits of 'japonica' such as bacterial blight resistance and high yield. These varieties proved specially good to Indian climate but unfortunately were not accepted by Indian people due to sticky grains arising from a low amylose content. With the use of gamma rays treatment, a strain of Taichung 65 with high yield and grains with a high amylose content and non-sticky nature has been developed.

Rice is grown in nearly 35 million hectares in India but greatly suffers from poor yield nearly 1,100 kg./ha. in contrast to over 4 tonnes/ha. in Japan. Indian rice have weak and tall straws which make cultivation and application of fertilisers difficult. To overcome this difficulty, fertiliser responsive and photo-insensitive varieties have been developed using dwarfing genes in the mutant of the indica variety—Dee-gee-woo-gen. Taichung Native-1 is the first outstanding dwarf variety developed by crossing a tall *indica*, Tsai Yuen-Chung with Dee-gee-woo-gen. It has a high degree of resistance. I. R. 8 is another variety derived by a cross between a tall *indica* variety 'Peta' and Dee-gee-woo-gen. It is high yielding producing upto 10 tons/ha. and is specially successful in many countries in South East Asia including India.

Wheat is a common crop in India cultivated nearly in 13 million hectares of the land. It has always suffered with poor yield from last 30 years. The basic reasons for its poor yield were (i) the tall plant habit which results in lodging in fertile soils and (ii) the rapid rise in temperature in February-March which imposes a ceiling on grain development and creates problems of soil and atmospheric drought.

M. S. Swaminathan (1963) brought Mexican wheat to India which offered remarkable yield in Indian climate but he found that Mexican varieties could not easily be sold to Indian consumers because of grain colour. The grains were red. He, therefore, decided to initiate 'Crash' breeding programme to change the colour of Mexican wheat while maintaining the high yield and other good agronomic characteristics. While using gamma-rays treatment by G. Varughese and M. S. Swaminathan (1967) a few plants containing the desired amber seeds were discovered. Later in three and half years, the induced mutant variety **Sharbati-Sonara** was released to growers. It was readily accepted. This breeding programme was carried out at Indian Agricultural Research Institute, New Delhi. What was the most important in Mexican wheat was that they were dwarf spring varieties with a 'Norin' dwarfing genes developed by Norman E. Borlaug.

However, some of the important improved varieties of wheat are as follows:

1. **Lerma Rojo 64-A** : Late variety with a duration of 153 days, single-gene dwarf, high degree of yellow rust resistance, grains red and soft.

2. **Sonara 64** : Early variety with a duration of 133 days; shows promising results even in rotation with maize, rice, sugarcane, potato; susceptible to yellow rust hence recommended to grow in restricted areas, high yield upto 60 tonnes/hectare.

3. **Sonalika** : Derived from selection made from S. 308 which arose from a Mexican cross involving 'Norin 10 B'; single-gene dwarf, resistance to all three rusts.

4. **Safed Lerma** : Single-gene dwarf, derived from a Mexican cross (Norin 10 B) when back crossed thrice to Lerma Rojo 64-A; grains white and semi hard.

5. **Kalyan Sona** : A selection form of S-227 which is derived from the cross between Penjamo Sib and Gabo 55; two-gene dwarf, profuse tillering capacity; resistance to brown rust; high yield, amber grains; bread-making or chapati (unleavened) properties good; resistance to brown rust.

6. **Chhoti Lerma** : A selection form of S-331 derived from Mexican cross; two gene dwarf; resistance to lodging and all the three rusts ; grains white.

7. **Shrabati Sonara** : Resembles to Sonara—64; bold, amber, and attractive seeds containing about 16·5% protein in contrast to about 14% in Sonara 64; gives a higher loaf volume per 100g. of flour than Sonara 64 and Lerma Rojo 64-A; early variety with a duration of 120 days and yield over 6 tonnes per hectare.

GENERAL QUESTIONS

1. What is mutation ? How is mutation proposed by de Vries is different from its recent concept ? Write critically.

2. Discuss the various methods used for detection of mutation.

3. With the help of suitable recent studies, discuss briefly the mechanism of induced mutation.

4. What are spontaneous mutations and how are these produced in nature ? Give a critical thought to this respect.

5. Write an essay on role of mutation in plant breeding or agriculture.

6. How induced mutation proved helpful in the improvement of important crops ? Discuss.

■ ■ ■

CHAPTER 16

GENETICS OF NEUROSPORA

(Genetic Analyses and Biochemical Genetics)

Neurospora, an ascomycetous fungus, has been found quite suitable organism that offers exceptional opportunities for genetic studies. It has been considered for a long time as an ideal tool for genetic research, perhaps due to following reasons.

1. It has a brief life cycle and the main plant body is haploid.
2. It is well adapted for investigation on a mass production scale.
3. It is readily propagated by asexual reproduction.
4. It provides large number of population of a given type for a little trouble.
5. It can be easily cultured on a pure chemical medium, so controlled and biochemical experiments are easily conducted. Thus it provides a great advantage for physiological research.
6. Its life cycle permits to study exceptionally close genetic analyses.
7. Its fruiting body consists of asci, in each of which eight linearly arranged haploid ascospores are produced. The arrangement and number of ascospores in each ascus are of great significance as regards to study segregation of characters.
8. All products of single meiosis are retained within a structure called ascus. It obviously avoids confusion with the products of other meiosis.
9. The ascospores are the products of meiosis which attains a definite position in an ascus and a particular ascospore can be referred to the actual position of meiosis.

LIFE CYCLE

Genus *Neurospora* consists of several species but its species *N. crassa* has been thoroughly worked out from genetic investigation point of view. The main plant body of *N. crassa* is thread-like called mycelium which is haploid ($n=7$). It may reproduce both by asexual and sexual means. During asexual reproduction, unicellular spores called conidia are produced terminally. Asexual reproduction is based on mitotic divisions of haploid nuclei. The conidia are dispersed by wind each when fall on suitable medium germinates to form a new mycelium.

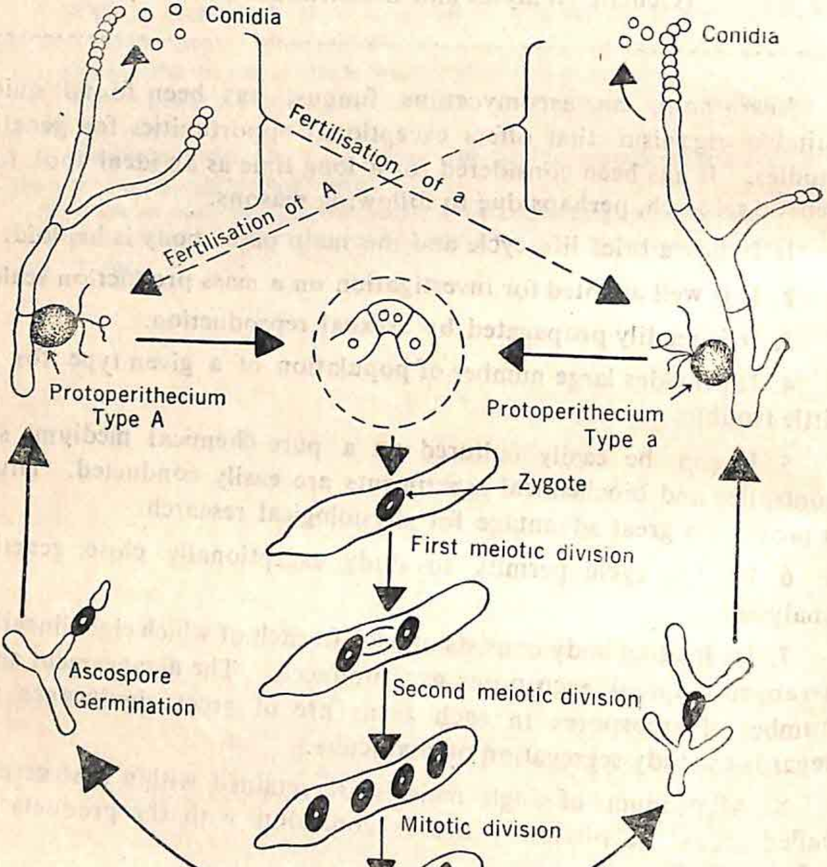


Fig. 16.1 : Diagrammatic representation of life cycle of *Neurospora crassa.*

Sexual reproduction occurs only when cells of opposite mating type unite, i.e., it involves a fusion of nuclei from mycelia of opposite mating types. These mating types are designated as *A* and *a* which are determined by members of an allelic pair. After fusion of two nuclei zygote is formed which constitutes the sole sporophytic stage in this fungus. It soon undergoes a meiotic division and produces a narrow sac-like structure called ascus. Its narrowness offers a great advantage in the orientation of ascospores within it. That is to say that the first division of meiosis occurs in tandem and the resultant nuclei cannot slip past each other. The nuclei remain in the order in which they were produced. The meiosis is usual that results in the formation of four nuclei. Thus, two nuclei at one end of the ascus are derived from one of the first division nuclei while the two nuclei at the other end of ascus are derived from other of the first division nuclei. Now each nucleus divides mitotically but again in tandem so that each meiotic product is represented twice within the ascus. Each nucleus differentiates into an ascospore. An ascus contains four pairs of ascospores, each pair representing one of the products of meiosis. The members of a pair of ascospores are genetically alike. These ascospores on rupture of ascus are released and each germinates to produce a haploid mycelium.

GENETIC APPLICATIONS OF NEUROSPORA

1. Inheritance Test in *Neurospora*

This may also be referred as the inheritance of single gene difference or the genetic events concerned with a first or second division segregation. Beadle (1947) conducted an experiment to analyse the basis of first-division segregation within an ascus of *Neurospora*. He selected two variant forms of *N. crassa*, one showing growth as a spreading mycelium (normal type) and other showing a button-like or colonial type of growth (colonial type). On mating asci were developed. These asci were dissected and careful separation of ascus then of ascospores was made. It was expected that upon germination of the eight ascospores resulting from such a cross, four will show a normal spreading growth and other four will show the colonial growth provided these characters are determined by a single gene. Similar results were obtained.

The experiment has been elucidated in the fig. 16.2. The normal strain has a gene (+) on one chromosome that determines spreading growth while colonial strains has a gene (*c*) that determines colonial growth. As is shown in the figure, the fusion nucleus contains two homologous chromosomes, one bearing normal gene (+) and other colonial (*c*). When meiosis occurs, these two homologous chromosomes pair and form a tetrad. At first division, the

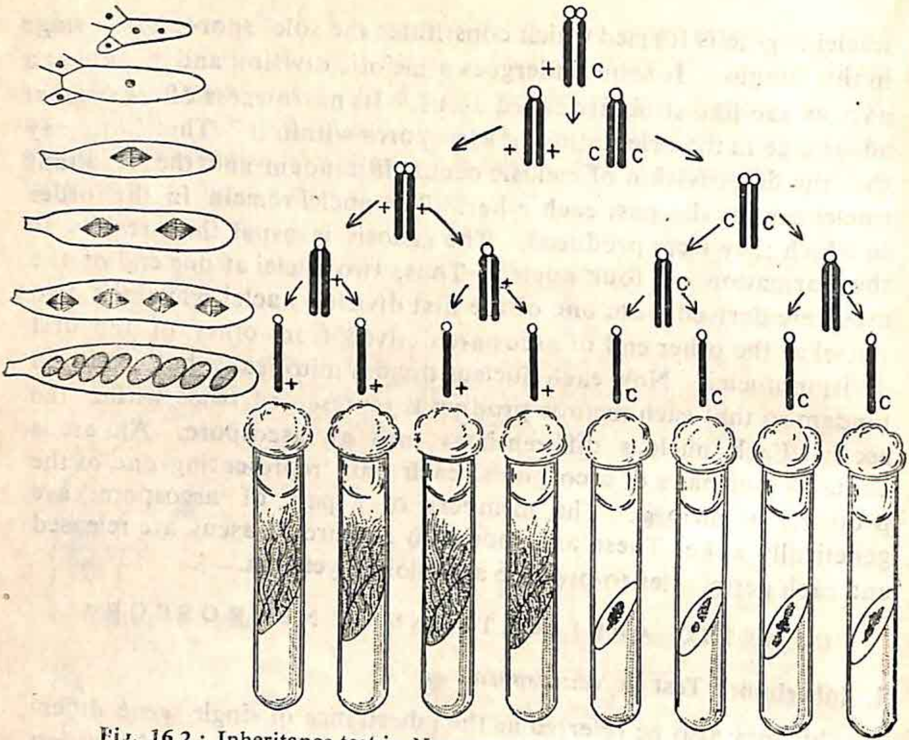


Fig. 16.2 : Inheritance test in *Neurospora* explaining the basis of first division segregation within an ascus. Gene *c* determines colonial type of growth while its wild type (+gene) determines spreading mycelial growth. On the left, sequence of ascospores formation within an ascus are shown (see text).

two alleles (+ and *c*) segregate from each other and the replicated members of the pair are drawn to opposite end. Soon the second mitotic division occurs in tandem and the two nuclei with (+) gene and two with (*c*) gene are formed. Each nucleus now undergoes a mitotic division to give four nuclei having the (+) gene and other four nuclei having the (*c*) gene.

Certain conclusions can be drawn from this experiment. While it illustrated the important general principle that if any trait in a haploid organism is controlled by a single gene, it must show a one to one (or here 4 : 4) segregation in progeny, it also explains that both the parents share equally in the formation of progenies (or there is equal genetic contribution by each parent) and each homologous chromosome shows equal replication.

Considering the results of the experiment, the spore order may be represented simply as + + + + c c c c or c c c c + + + + depending upon whether the chromosome carrying gene (+) lines up to the right or to the left of its homologue at the first division of meiosis. These results will infact be seen if no cross over occurs between two genes. There have been some variations from the expected orientation, i.e., linear arrangement of 4 : 4. Spore combination like 2 : 2 : 2 : 2 are sometimes found. Their orientation may be

++ cc ++ cc
cc ++ cc ++
++ cc cc ++
cc ++ ++ cc

Beadle studied these variations in separate experiments and

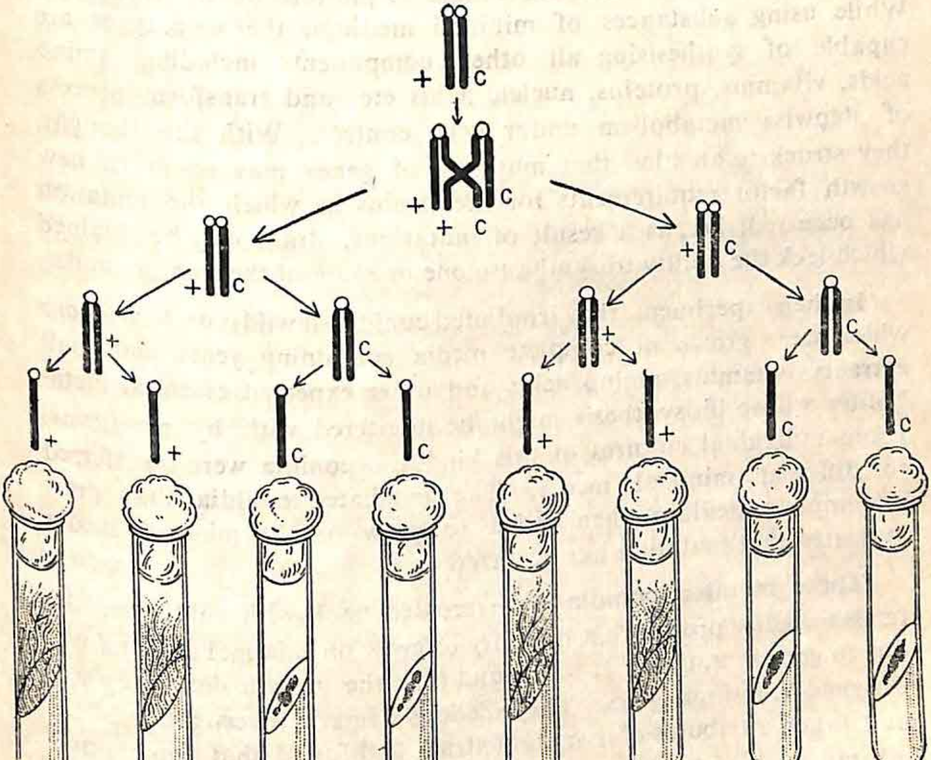


Fig. 16.3 : Inheritance test in *Neurospora* explaining the second-division segregation due to exchange between chromatids that occurs between gene locus and the centromere.

accounted 2 : 2 : 2 : 2 orientation due to segregation occurring at second division of meiosis as is shown in fig. 16.3. It happens due to occurrence of a crossing over at first division resulting their segregation at the time of second division. When crossing over occurs between a gene and the centromere of its chromosome, segregation does not occur at the first division of meiosis but is deferred to the second division.

2. Detection of Biochemical Mutations

Beadle and Tatum (1947) studied extensively *Neurospora crassa* and used it as a tool for the analysis of various biochemical mutants. The fungus can be grown on a culture medium containing a few simple salts, some sugar and biotin. The medium may be referred as minimal medium and such fungus is phototroph or wild type. While using substances of minimal medium, the wild types are capable of synthesising all other components including amino acids, vitamins, proteins, nucleic acids etc. and transform process of stepwise metabolism under gene control. With this thought, they struck to an idea that mutation of genes may result in new growth factor requirements for the strains in which the mutation has occurred, i.e., as a result of mutations, strain may be obtained which lack the ability to synthesise one or more of these compounds.

In their experiment, they irradiated conidia of wild type *Neurospora* which were grown in complete media containing yeast and malt extracts, vitamins, amino acids and other expected essential metabolites whose biosynthesis might be interfered with by mutations. From individual cultures of this kind, the conidia were transferred to different minimal media. The irradiated conidia that grew in complete medium when failed to grow on the minimal media indicated that mutation has occurred.

These mutated conidia were crossed back with wild type. Its further studies proved that inabitity to grow on minimal medium was due to gene mutation. It was found that the growth deficiency was assigned to a single gene. They made systematic investigation of the nutritional attributes of a mutant strain and found that some single substance added to the minimal medium satisfied the growth requirement. They found a number of different biochemical mutants produced in *Neurospora*. Some of them were identified by the compounds they require for growth such as (i) thiamine, pyridoxine,

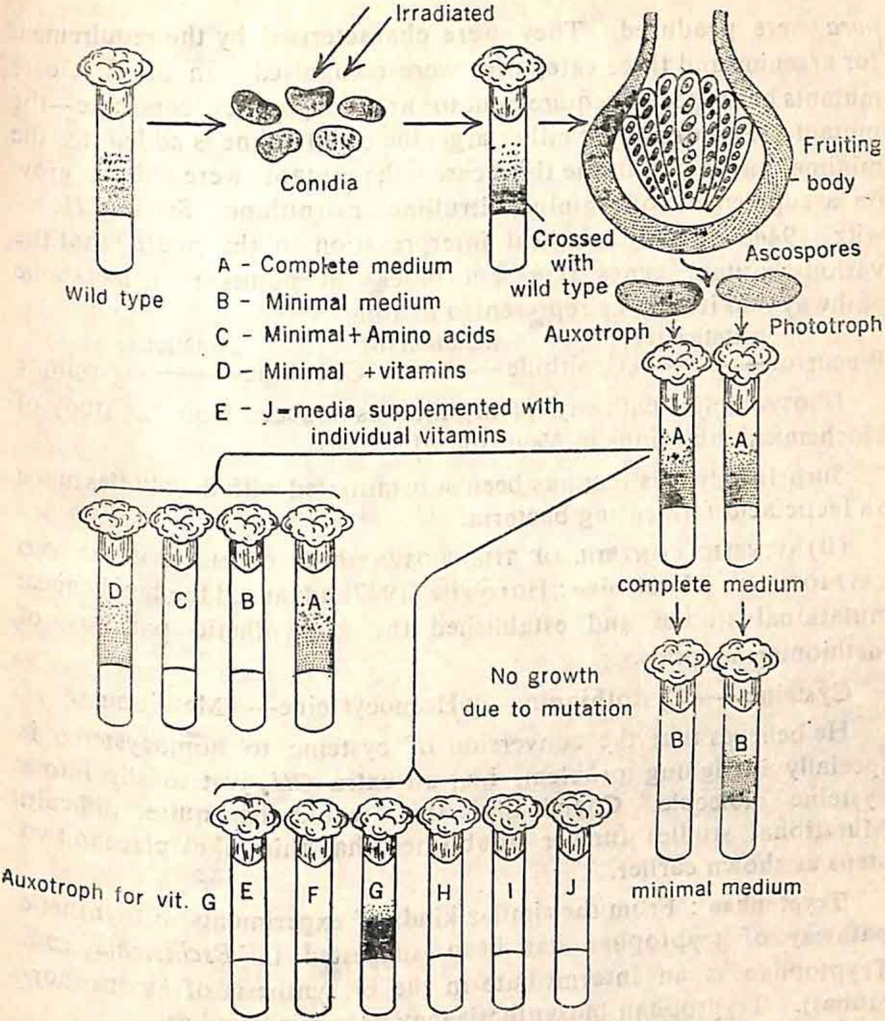


Fig. 16.4 : Detection of biochemical mutations in *Neurospora*.

pantothenic acids, inositol, nicotinic acid, choline, riboflavin (all vitamins); (ii) arginine, leucine, lysine, tryptophan, valine, serine (all amino acids) and (iii) adenine, pyrimidine, succinic acid etc. as other compounds.

This method of study gave many fabulous results such as establishment of different biosynthetic pathways occurring under genetic control. A few are discussed here.

(A) GENETIC CONTROL OF THE BIOSYNTHESIS OF ARGENINE : With the use of mutagens, several genetically different mutant strains of *Neuros-*

pora were produced. They were characterised by the requirement for argenine and three categories were recognised. In the first case mutants have specific requirement for arginine, in the second case—the mutant grew normally if either argenine or citrulline is added to the minimal media and in the third case—the mutants were able to grow on a supplement of arginine, citrulline or ornithine. Srb and Horowitz (1944) offered a logical interpretation to this finding that the various mutant genes represent blocks at points in a metabolic pathway and it may be represented as follows:

$$\text{Precursor}\xrightarrow{\text{mutation (c)}}\text{Ornithine}\xrightarrow{\text{mutation (b)}}\text{Citrulline}\xrightarrow{\text{mutation (a)}}\text{Arginine}$$

[Biosynthetic pathway of arginine as deduced from the study of biochemical mutations in *Neurospora*]

Surprisingly this fact has been substantiated with the studies made on lactic acid fermenting bacteria.

(B) GENETIC CONTROL OF THE BIOSYNTHESIS OF METHIONINE AND TRYPTOPHAN : **Methionine** : Horowitz (1947) advanced the biochemical mutational studies and established the biosynthetic pathway of methionine as follows :

Cysteine→Cystothionine→Homocysteine→Methionine.

He believes that the conversion of cysteine to homocysteine is specially intriguing problem, i.e., an extra CH_2 just to slip into a cysteine molecule. Chemically, this would be quite difficult. Mutational studies further established that this takes place in two steps as shown earlier.

Tryptophan : From the similar kinds of experiments, biosynthetic pathway of tryptophan has been suggested in *Escherichia coli*. Tryptophan is an intermediate in the biosynthesis of auxins (hormones). Tryptophan biosynthesis may be summarised as

Anthranilic acid→Indole-3 glycerol→Tryptophan

[Tryptophan→Tryptamine→Indolepyruvite→Indole acetic acid (IAA)].

GENERAL QUESTIONS

1. Write a short account of biochemical genetics.
2. Biochemical genetic studies has given solution to many problems related to metabolism. Discuss it with the help of suitable examples.
3. Write short notes on—(i) Life cycle of *Neurospora*, (ii) Inheritance test in *Neurospora* and (iii) Biochemical mutations.

■ ■ ■

CHAPTER 17

THE GENE

Mendel was almost a unique personality to establish the relational inheritance that offsprings are necessarily related to the parents. He stated that in the resultant progenies recombinations occur. For his principle of recombination, the principle of segregation is prerequisite. While he understood the mechanism of heredity, his most outstanding contribution was the realisation of hereditary differences being caused by particular determiners which are now called genes. Genes are, therefore, the elements by means of which the transmission mechanism of heredity occurs.

The Relation of Genes and Chromosomes : Interestingly when the paper of Mendel appeared, Haeckel also published his observations and proposed that the nucleus is the part of cell and is responsible for heredity. Since then, the subject cytology underwent great changes and the behaviour of chromosomes during mitosis, meiosis and fertilisation was studied by Weismann, Strasburger and others and the idea of relation of chromosome to heredity was developed by 1900. The detailed accounts in this respect were compiled in the Wilson's book entitled "The Cell".

McClung (1901) was the first man to establish relationship between a particular chromosome and specific inherited character when he observed in *Orthoptera* one strongly heterochromatic chromosome (sex-chromosome) in male and not in female. He proposed that heterochromatic chromosome containing sperms are male producing. By 1910, Morgan reported cases of sex-linked inheritance. Such studies revealed that chromosomes are sole factors of heredity and the 'elements' or 'genes' locate on them.

Defining the Genes : The term 'gene' was coined by Johanssen (1909). He defined that "any particle to which the properties of a Mendelian factor may be attributed is called gene". Morgan (1925) defined "Any particle in a chromosome which is distinguishable from other particles by their crossing over or mutation." Muller and Prokofieva (1935) defined that "A minimal particle of the chromosome which can be separated from other particles by X-ray breakage." Some of the other important definitions of the gene are as follows:

(i) A gene is a unit of chromosomal structure not subdivisible by chromosomal breakage or crossing over.
(ii) A gene is unit of mutation.
(iii) A gene is unit of biochemical reactions.
(iv) A gene is unit of physiological actions.
(v) A gene is unit of heredity.
(vi) Gene is the ultimate unit of recombination.
(vii) Gene is the ultimate unit of self-reproduction.
(viii) Genes are descrete, self-propagating parts of the cellular organisation.
(ix) Gene is the ultimate unit of function.

In the simplest form defining the gene as the ultimate unit of function is most safe because all the reactions within the living organisms are more or less governed by genes. Genes may also be considered as the genetically functional segment of a *DNA* molecule.

Nature of Genes : Gene is a unit of chromosomal structure and not subdivisible by chromosomal breakage or crossing over. These are descrete, self propagating parts locating in the chromosome and form definite zones in it. These zones appear as bands, that resemble with spectrum band, in the multitene chromosome of *Drosophila* salivary gland. These bands indicate the position of genes. Thus, genes are situated in the chromosomes, each separated from the other by non-genic material.

Genes are arranged end to end serially. The behaviour of chromosomes during cell division such as longitudinal pairing, crossing over, linkage and chiasmata formation indicate the arrangement of genes as above. In a chromosome, the number of genes is definite but are always numerous. Adopting the map drawing technique of genes, their number in many organisms has been studied. In a lily plant, 2000 to 2500 chromomeres per chromosome and in *Drosophila melanogaster*, 5000 to 6000 chromomeres per chromosome have been noted. Each chromomere may contain number of genes. Certain bacteriophages, which are often considered as smallest living organisms, have been found to contain minimum number of 75 genes per individual. The fruit fly, *Drosophila*, has about 10,000 genes in a normal body cell.

The use of word 'gene' is a bit confusing when its composition, structure, behaviour and functions are visualised, and its use to designate as any specific piece of genetic material would lead to several new problems. There has been a great deal of conflict during last many years due to the fact that the same words were being used with different meanings by different authors. The genes cannot be visualised any more as well separated beads on a string. They are part of a much more continuous structure.

Size of Genes : Genes cannot be seen with the help of compound microscope. It may, however, be assumed that a gene must be 'hit' by an ionising particle in order to be caused to mutate by an ionising radiation. When calculated by 'largest size estimation' the diameter of one gene is to be something like 6 millimicrons (0·000006 mm) and its molecular weight around 100,000. Similarly the 'upper limit size' can be calculated by estimating the number of genes per chromosome. This gene size is actually the maximum size. In the *Drosophila* the bands in the chromosome represent the location of gene. There are about 647 bands and 1000 genes in *x*-chomomosome. When volume of gene estimated by 'upper limit size', an average gene is 20 *m*μ to 100 *m*μ. This will give a molecular weight of about 25 million.

However, these methods are mere calculations and geneticist like Muller does not believe in them.

Genes is the Ultimate Unit of Recombination : This concept visualises that genes are those parts of chromosome between which crossing over takes place. The recombination of two genes and separation of non-allelic genes have been observed in *Drosophila* and *Zeamays*. However, studies on bacteriophages suggest that the recombination can occur among the alleles of given locus. Benzer (1955) noted the linear arrangement of genes along the linkage map which are separable through recombination in bacteriophage. Thus, he proposed that recombination can take place within a gene. It is because functional genetic units are composed of smaller recombintional and mutational elements. This concept does not fit with *Drosophila* where the functional, recombinational and mutational unit is one and is the same physical unit.

Gene is the Ultimate Unit of Function : Sinnott et al (1958) proposed that gene is the ultimate unit of function. They found that when studies in the line of genetics are made at molecular level, the gene becomes the basis of all the activities. It thus means that gene is the site of all the vital activities. Every gene has its peculiar type of chemical constitution that not only relates but also governs the other functions of cells.

Bazer (1962) recognised three functional units to correlate the physiological aspects of gene.

1. *Recon* : It is referred to the smallest unit segment of *DNA* that undergoes crossing over and recombination. It may even consist of one or two nucleotides as in certain microbes. It has, so far, little bearing on studies of protein synthesis.

2. *Muton* : It is the smallest unit the alteration of which results in mutation. In its smallest expression, muton represents a change in a pair of nucleotides. So there is a space for many mutons within the locus of an enzyme. It has important bearing on protein synthesis, for it is directly related to the smallest modification of the genetic material which causes a change in the structure of the protein produced, i.e., any change in a base triplet will alter the message carried by codon and will code for other amino acid.

3. *Cistron* : Cistron is, in fact, the unit of function and is the largest sub-unit of *DNA* material. It can be defined as a piece of genetic material which must be present as a unit to accomplish its function. In each cistron unit, there can be more than 100 muton units or as many as 30,000 nucleotides. More clearly, cistron is a segment of genetic material which specifically controls the synthesis of this particular protein. It ceases to fulfil this function if it is fragmented.' Cistron is thus a unit of *DNA* which accomplishes one indivisible function required for the synthesis of that specific protein.

Considering the significance of these terms, certain new terms have also been recently introduced such as

Complon : A unit of complementation (rather a new term for cistron). Complon controls any alternation or deficiency in a polypeptide chain.

Replicon : A unit of replication.

Codon : A triplet of messenger *RNA*.

Transcripton : A unit that contains a block of several enzymes and transcribes the message.

Operon : A unit that operates a specified function. It contains a block of several enzymes.

Stability of Genes : Genes are considered as extremely stable structure. They are not even subdivisible by chromosomal breakage or crossing over. They are found in unchanged form in a cell from generation to generation. The stabitity of genes is studied in terms of half life of a gene, i.e., the time in which 50% of the genes would be expected to mutate. Muller (1950) calculated the gene stability in *Drosophilu* that a gene has half life of 10^3 to 10^4 years. In maize, *R* gene mutates at the rate of 492, the *S* gene in every 10^6 gametes, the *I* gene in 106 and *Wx* not at all in every $10^5 - 10^6$ gametes. Stability of gene can be influenced by various radiations, chemicals etc. These have been described in mutations.

Position effect: Two examples of position effect are well known—position effect of genes in *Drosophila* and $A_c - D_s$ effect in maize.

(i) *Position effect in Drosophila* : The presence of bar eye is known commonly in *D. melanogaster*. This phenomenon was studied by Sturtevant. He found that the development of bar eyes basically depends upon the position of gene and not on rest of genotype. His explanation was based on the fact that when the

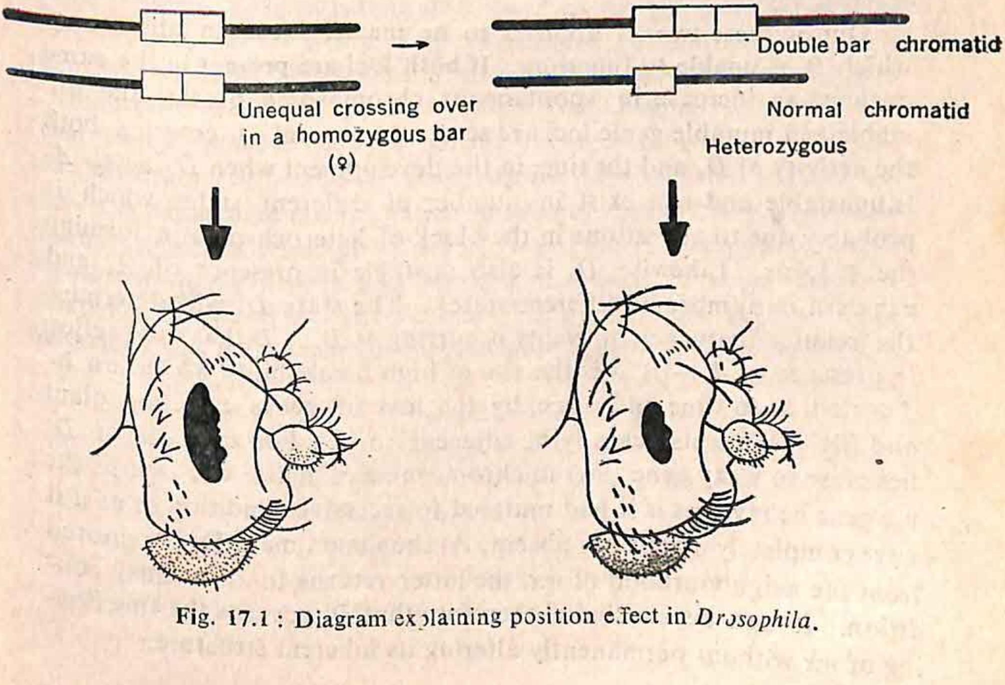


Fig. 17.1 : Diagram explaining position effect in *Drosophila*.

normal gene is duplicated, it causes reduction in the number of facets in the eye of the fly (bar eye). It was further confirmed by managing unequal crossing over resulting into three genes to be in the homologue and due to which the fly with double bar eyes was produced (fig. 17.1). Since in such crosses, there has been no change in the number of genes and the change is only in the position, it can be concluded that gene has got its particular effect at definite position. It can be further visualised that the function of the gene is dependent not only upon its particular properties alone but also upon the genes present next to it in the chromosome.

(ii) *A_c—D_s position in maize* : Out of her many contributions the establishment of A_c—D_s position in maize is the most remarkable contribution of Mc Clintock (1950, 53). She demonstrated that the expression of gene is closely related to chromosomal organisation. While working on mutant strain of maize, she noted the relative control of genic action of two loci. To one she referred as Activator (A_c) and to other dissociation (D_s). Earlier, these were referred as individual genes (as they exhibited Mendelian inheritance) which show crossing over with other genes and were mutable. But these are referred as *loci* since evidence indicates that those are the blocks of heterochromatin.

Out of these two A_c appears to be master locus in absence of which D_s is unable to function. If both loci are present in the same nucleus, an increase in spontaneous chromosome breaks and unstable and mutable genic loci are seen. In general, A_c controls both the activity of D_s and the time in the development when D_s acts. A_c is unastable and can exist in number of different states which is probably due to alterations in the block of heterochromatin forming the A_c locus. Likewise, D_s is also unstable in presence of A_c and can exist in number of different states. The state D_s will determine the relative frequency of events occurring at D_s. D_s has dual action in presence of A_c—(i) it is the site of high breakability which can be detected, as to time and place, by the loss of genes affecting plant and (ii)—D_s affects genes lying adjacent to it. For example, if D_s lies close to waxy gene (*wx*) in chromosome of maize endosperm the *wx* gene behaves as if it had mutated to recessive condition or as if it were completely inactive or absent. At the same time if D_s is removed from the neighbourhood of *wx*, the latter returns to its normal condition. It can be concluded therefore that D_s governs the functioning of *wx* without permanently altering its inherent structure.

Gene Action : Broadly two important characteristic functions are visualised.

(i) *Self reduplication* : Each gene makes an exact copy of itself by utilising food material available to all genes.

(ii) *Governing action* : It manages the reaction running outside the nucleus by means of its complex activities, i.e., Gene forms and gives off substances which influences specific reaction, firstly inside the cell and subsequently elsewhere in the organism. In this relation the basic function of gene is to elaborate highly specific primary products important with the physiology of cells. Genes regulate the specific biosynthesis of proteins otherwise used in the composition of enzyme and thus regulating the key reactions in the organism.

Physical Studies and Chemical Composition : Efforts made on visualising the physical studies of genes indicate that genes are, probably, no smaller than protein molecules having a molecular weight of 100,000 and no longer than average size of viruses.

Chemically, genes are nucleoproteins, a complex of nucleic acid and protein. It has been discussed separately

Genes at the Core of Crime : W. H. Price and P. B. Whatmore have made extensive studies and are the pioneer workers to propose that in man the criminal disposition is inherited and the core of the crime is gene. They substantiated this fact on the basis of several observations. There are records of persons committing crimes as their hobby. The tendency of crime occurs when one or more extra chromosome are found in each cell. The criminal tendency has been found increased in the person having one or more extra sex chromosomes, e.g., normal man has xy $(22+xy)$, and female xx $(22+xx)$ while male mental patient possesses, $xxyy$ $(22+x\ x\ y\ y)$ and insane (senseless) patient—$x\ y\ y$ $(22+x\ y\ y)$. These patients were emotional, irresponsible and showed tendency of crime such as murder, assault and sexual offences. They concluded that criminal behaviour was due to extra y-chromosome present in these patients.

■ ■ ■

CHAPTER 18

ONE GENE—ONE ENZYME HYPOTHESIS

From the time Garrod drew attention to several hereditary abnormalities of man such as excretion of unusual substances in the urine and changes in pigmentation, which are inherited from parent to progeny in a way as if these are controlled by single recessive alleles, a clue for genic role in relation to physiogical abnormalities sprang up. These examples were studied in details.

1. Alcaptonuria

In a classical example of alcaptonuria in which case patients excrete homogentisic acid that gives rise to a black pigment, it was observed that when the acid is fed to alcaptonuric, it can be recovered in the urine whereas it disappears in a normal man. This fact was elucidated as early as 1914 by Gross that the normal human serum destroys homogenistic acid while alcaptonuric serum cannot.

$$\text{Normal—Homogentisic acid} \xrightarrow{\substack{\text{gene A} \\ \text{(enzyme)}}} \text{Maleylacetoacetic acid}$$

$$\text{Alcaptonuric—Homogentisic acid} \xrightarrow[\text{(no reaction)}]{\substack{\text{gene A} \\ \text{(no enzyme)}}} \text{Homogenetisic acid}$$

Clearly, the alcaptonurics suffer from a metabolic disorder and there is a failure of some enzymic process. In view of the fact that this disease is controlled by a single gene, it appears that gene controls enzymic process in some way.

2. Floral Pigmentation

Similar observations were recorded by Onslow and Bassett (1914) while studying inheritance of floral pigmentation in cape primerose, *Streptocarpus*. It was later on brilliantly studied by Scott-Moncrieff (1930, 1939) and Lawrence (1950). While analysing the chemical structure in relation to genetic control, they found that though different pigments were chemically closely related and belonging to the same class—anthocyanidins, each strain was found to contain essentially one single pigment. The various pigments differ from each other by the presence of a few glycosyl or methoxy groups on the hydroxyls of the basic anthocyanin structure. Studies made on the pattern of inheritance of pigments revealed that as if these were controlled by three pairs of alleles, with one dominant and one recessive in each pair, *Oo*, *Rr*, *Dd*. In case of genic combination *ord*, the pigment synthesised was pelargonium-3-pentose-hexoside (salmon). When recessive *r* is replaced by dominant *R* (*oRd*), a substitution of a methoxy group at position 3′ occurred (rose). Likewise in presence of *ORd*, two methoxy groups at position 3′ and 5′ appeared (mauve) and when dominant *D* appeared (*ORD*), hexose was found at position 5, giving a 3, 5-dihexoside in place of the 3-pentosehexoside (blue).

The example of inheritance of pigment in *Streptocarpus* reveals that individual genes thus correspond to certain chemical operations in the synthesis of pigments. Clearly the genes control well defined

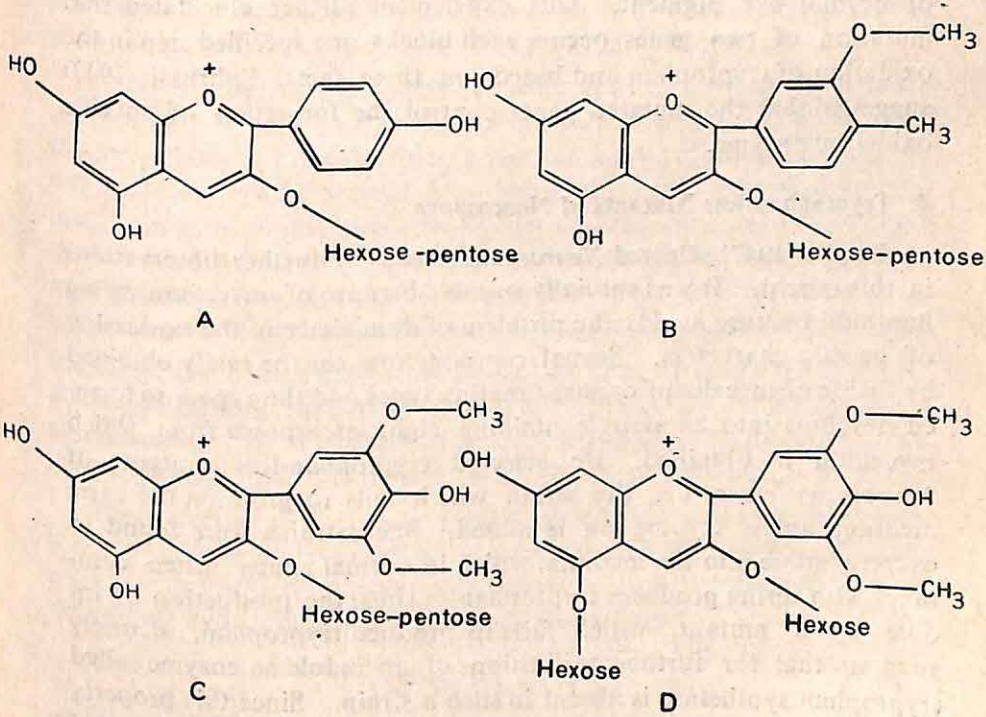


Fig 18.1 : Floral pigments from four *Streptocarpus* varieties as influenced by different genes—(a) formed with *ord*, (b) with *oRd*, (c) with *ORd* and (d) with *ORD* (see text).

steps in the enzymic process involved in the synthesis of the petal pigments.

3. Eye Pigments in Drosophila

Further information on the genetic control of anabolic processes was provided by Beadle and Ephrussi. They found that development of the eye pigments in *Drosophila* is due to presence of two types of coloured granules, the red and brown in the pigment cells surrounding the ommitidia. In a series of mutants such as vermilion, cinnabar, scarlet etc., they carried out transplantation experiments. On transplantation of eye disks of vermilion or cinnabar larvae into abdomen of wild larvae, they observed that mutant eye disks developed wild type flies supplied substances which are required for the development of normal pigment. They further observed that the presence of substance kynurenine causes the change from vermilion to cinnabar and presence of hydroxykynurenine from cinnabar to wild. Both these compounds are intermediates in the biosynthesis of normal eye pigment. This experiment further elucidated that mutation of two genes occurs each blocks one specified step in the oxidation of tryptophan and based on these facts, Ephrussi (1942) suggested that the mutated genes control the formation of specific oxidation enzyme.

4. Tryptophan-less Mutants of Neurospora

Beadle (1947) selected *Neurospora crassa* for further observations in this regard. It was specially suitable because of mycelium being haploid in nature avoids the problem of dominance in the expression of genetic characters. Sexual reproduction can be easily obtained by fusion of mycelia of opposed mating types and the zygote so formed develops into an ascus containing eight ascospores from which mycelium is obtained. He selected tryptophan-less mutant of *Neurospora crass*, i.e., the strain which fails to grow on the basic medium unless tryptophan is added. Such strains were found to excrete indole into the medium, which in normal course when combines with serine produces tryptophan. Thus, the production of indole by a mutant, which fails to produce tryptophan, strongly suggests that for further utilisation of an indole an enzyme called tryptophan synthetase is absent in such a strain. Since this property is inherited, it can he concluded that the hereditary defect is located at the level of one well defined chemical reaction catalysed by a known enzyme. Thus, the genetic analysis of tryptophan less mutant of *N. crassa* further shows that this tryptophan requirement is inherited as a single mendelian character and a well defined chemical reaction is governed by a single gene.

5. Sickle-cell Anaemia

The example of sickle-cell anaemia is extremely interesting since here it is easy to isolate and to purify the proteins involved in the process. The disease is also popularly called hereditary blood disease of man. It is frequently found among Nigerian people. The red blood cells in diseased human beings show abnormal behaviour, i.e., when oxygen tension is low, the blood cells become twisted and under the microscope these look somewhat like sickles. Neel (1949) established that disease is inherited in a Mendelian pattern. At the same time, Pauling and co-workers (1949) observed that the blood cells of individuals carrying the sickle cell trait contain a haemoglobin which can be separated from normal haemoglobin by electrophoresis. The sickle-cell haemoglobin is little less negatively charged and is comparatively less soluble at low oxygen pressure.

Further genetic studies made revealed that sickle cell behaviour of red blood cells is found in the individuals who are homozygous for the sickle-cell gene and it is a consequence of inborn defect in haemoglobin. Ingram (1956,-'57) has been able to compare amino acid sequence of haemoglobin *S* (sickle-cell) and haemoglobin *A* (normal adult individual) and also of haemoglobin *C* and haemoglobin *G* (the other haemoglobin types). He observed the amino acid sequence of terminal part of the *B*-chain in haemoglobins as follows:

Haemoglobin *A*	Val—His—Leu—Thr—Pro—Glu—Glu—Lys
Haemoglobin *S*	Val—His—Leu—Thr—Pro—*Val*—Glu—Lys
Haemoglobin *C*	Val—His—Leu—Thr—Pro—*Lys*—Glu—Lys
Haemoglobin *G*	Val—His—Leu—Thr—Pro—Glu—*Gly*—Lys

These remarkable studies clearly show that mutation of a gene can result in the replacement of one amino acid by another in the protein. It has been further observed that such mutation does not induce any other change in the rest of the chains.

CONCLUSION

While considering the examples discussed earlier, this can well be concluded that the genetic material controls the nature and position of certain amino acids in the polypeptide chains. Based on these facts, Horowitz proposed **one gene—one enzyme** hypothesis as follows:

"A large class of gene exists in which each gene controls the synthesis of, or the activity of, but a single enzyme."

Concluding the hypothesis one gene—one enzyme hypothesis, Chantrenne (1961) states that "the gene responsible for the production of a given protein contains a set of instructions concerning the nature and positions of the amino acids in the polypeptide chain, perhaps a complete blue print of the polypeptide chains."

FEW COMPLICATIONS CONCERNING HYPOTHESIS

Recent findings have suggested that gene itself consists of two contiguous pieces of the genetic material, so called *cis* and *trans*. Such a concept, however, does not contradict the basic idea of one gene—one enzyme hypothesis provided one is ready to readjust the definition of the gene and enzyme adequately. There are still other conflicting experimental findings which invite reconsideration of the hypothesis. A few are discussed here.

(a) PLEIOTROPY : Pleiotropy has been reported in the higher organisms where in certain cases, a single mutation changes more than one morphological and physiological characters, i.e., one gene affecting more than one character. Later, this phenomenon was also observed in certain micro-organisms in which case certain genes have been found affecting more than one biochemical character.

Both these complications have been explained separately. In higher organisms, it is highly probable that one single biochemical deficiency in the zygote will have several effects in the future course of development. However, in micro-organisms, the explanation is more or less based on experimental facts. For example, the double requirement for methionine and threonine in a *Neurospora crassa* strain has been found that it is the result from a single genetic block in the pathway of homoserine synthesis which acts as a common precursor of both threonine and methionine, respectively (Horowitz, 1956).

(b) SUPPRESSION : Suppression phenomenon is rather more puzzling. Two types of cases are more complicated. In one case, suppression of a nutritional requirement by such a mutation which occurs in a region of genome completely unrelated to the locus has been observed. In other case, suppressor mutation was shown to open a pathway of biosynthesis alternative to one which was blocked.

In all such cases, suppressors affect the operation of enzymes or of enzyme systems. These facts do not conflict the basic idea of the hypothesis. It can, however, be visualised that final shape of the protein is not completely determined by the corresponding locus and that, factors which are independent of this locus may play a part in shaping the protein molecule.

(c) CONTROLLING GENETIC UNITS : The work of Jacob and Monad (1961) on the genetic control of β-galactosidase synthesis in *Escherichia coli* revealed that the production of the enzyme is controlled by three independent and separable genes *z*, *i*, *y*. The detailed study further elucidated the fact that the three loci (genes), however, execute three different functions. The *z* locus provides structural information for β-galactosidase enzyme and the *i* locus controls the actual production of enzyme. The third gene *y* regulates the synthesis of β-galactosidase indirectly by controlling the formation of a system which concentrates into the bacteria inducers of β-galactosidase synthesis. Thus, these facts are in perfect agreement with the concept of the hypothesis.

DISCUSSION

Though these findings could be explained satisfactorily in the favour of one gene—one enzyme hypothesis, yet they offer a prelude to recognise a fact that more than one gene may be found involved in the control of the activity of a single enzyme (e.g., *z*, *i*, *y* genes in *E. coli*). Similarly, further studies on the genetic control of the haemoglobin molecule in man revealed that two different genes control its structure. The study of the haemoglobin from mutant individuals reveals that in fact the different genes control the structure of the different polypeptide chains of the molecule, i.e., one gene controls the α-chain and other β-chain. These conclusions suggest that one gene-one enzyme hypothesis should be further modified to a one gene—one polypeptide hypothesis or one cistron—one polypeptide hypothesis. Incidently, the recent findings have further challenged this compromise because it has been recorded that not all genes are involved in the enzyme synthesis rather there is a class of genes, the regulatory genes, which regulates and modulates the action of enzyme-determining genes (also discussed in a separate chapter). Besides, not all the protein synthesised by the genes act as enzymes. Many genes produce proteins whose cellular function is other than enzymatic, e.g., protein synthesised by viral genes make up the coat of the virus. Thus, the validity of such hypothesis is further threatened.

GENERAL QUESTIONS

1. With the suitable examples write an essay on 'one gene one enzyme' hypothesis.

2. What is one gene one enzyme hypothesis ? Discuss it in the light of recent researches.

3. Write short notes on,

(i) Alcaptonuria, (ii) Floral pigmentation, (iii) Eye pigments in Drosophila, (iv) Tryptophan-less mutants of *Neurorpora*, (v) Sickle-cell anaemia and (vi) Molecular or hereditary diseases.

■ ■ ■

CHAPTER 19

EVIDENCES: DNA AS A GENETIC MATERIAL

[*Also*—Microbial Genetics]

Cytological evidences propose that the genetic information remains present in the chromosomes. But chromosomes are quite complex structure as regards to their morphology and chemical organisation. Chemically they consist of various types of proteins, *RNA* and *DNA*. Thus, either of the three in true sense can be the actual genetic substance.

Of the three substances, *DNA* is qualitatively constant from cell to cell within a species. And for each species also the amount of *DNA* per cell is almost constant. In gametes, it is present half the normal quantity of *DNA* and in polyploid forms, it shows relative changes in its amount. The triploid and tetraploid organisms contain *DNA* three and four times than that found in haploid cells, respectively. On these grounds, it can be visualised that most likely carrier of genetic information is *DNA*.

Various biological tests have been carried out on haploid microbes. Some of them have extended the most conclusive evidences in the support of concept that *DNA* is the genetic material carrying the potentialities of transmission of hereditary characters. A few examples are discussed here.

1. Bacterial Transformations

In *Diplococcus pneumoniae* (*Pneumococcus*), *Bacillus subtilis*, *Hemophilus* sp. (streptomycin sensitive) and several other species of bacteria, it is possible to select genetically distinct strains of same species and transfer the genetic traits from one to other strain by incubating cells of one with purified *DNA* isolated from other. This phenomenon was discovered by Griffith (1928) in *D. pneumoniae* and was referred as transformation in bacteria. The identification of transforming principle as *DNA* beyond question was done by Avery, McLeod and McCarty in 1944. Three examples of transformation are discussed here.

(i) TRANSFORMATION IN *D. pneumoniae* : The bacterium *Diplococcus pneumoniae* (*Pneumococcus*) occurs in several genetically distinguishable forms. One form is **S-III type** producing colony with smooth surface. It remains surrounded by a polysaccharide capsule with specific antigenic property. The strain is virulent as it causes pneumonia on being injected into an animal (or mice). The other form is **R-II type** which lacks capsule and produces a colony with rough surface. It has neither the virulence (avirulent) nor the serologioal specificity as found in S-III type.

Griffith used both these strains in his experiments on mice. He observed that mice injected with R II type did not suffer with pneumonia. Similarly when these were injected with heat killed virulent S-III type, these did not suffer with pneumonia. He further observed that when mice are injected with R-II type together with large quantity of heat killed virulent S-III type, symptoms of pneumonia disease appear. While examining the blood of mice it was found that both S-III and R-II types of bacteria were present in it. It led him to conclude that some of the R-II type have been transformed into virulent S-III type.

This kind of experiment was also carried out *in vitro*. R-II type cells were grown in a test tube with heat-killed S-III type and it was found that live S-III type cells were produced among the R-II type cells. Avery et al (1944) carried out another experiment and concluded that the presence of heat-killed S-III type in R-II type culture for the production of virulent S-III type is not necessary rather R-II type cells could be transformed in the presence of extract of S-III type. This shows that the presence of certain substance is needed for this transformation. Later, the substance was identified by them and was found to be *DNA*. Such experiments were further advanced to find out mechanism of such a phenomenon.

Mechanism of transformation : Herskowitz (1968) states that "Genetic transformation is a type of genetic recombination in which one single-stranded segment of *DNA* is substituted for another. The process involves binding, penetration, synapsis and integration of donor *DNA* in a recipient cell."

Binding : Bacterial *DNA* is at first bound reversibly to the cell surface at receptor sites. The binding property is observed only in *DNA* of high molecular weight. *DNA* becomes permanently bound to cell surface.

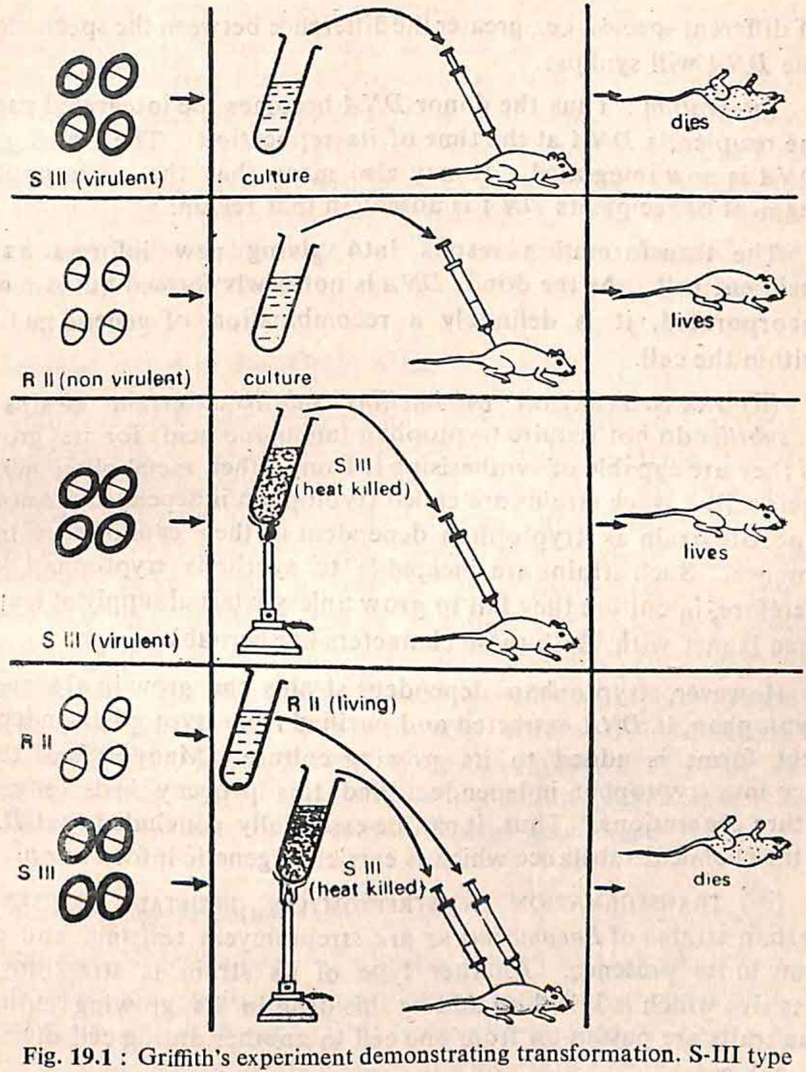


Fig. 19.1 : Griffith's experiment demonstrating transformation. S-III type is virulent and causes pneumonia on injecting while R-II is non-virulent and does not cause pneumonia.

Penetration : The permanently bound *DNA* penetrates the bacterium. It is believed that penetrating *DNA* must have a molecular weight of 5×10^5. It is now called donor *DNA*.

Synapsis : Along with the corresponding segment of the recipients *DNA*, the penetrated *DNA* pairs locus for locus. This phenomenon is called synapsis. The synapsis is relatively infrequent in the *DNA* of different species, i.e., greater the difference between the species lesser the *DNA* will synapse.

Integration : Thus the donor *DNA* becomes the integrated part of the recipient's *DNA* at the time of its replication. The transformed *DNA* is now integrated. It may also mean that the corresponding segment of recipients *DNA* is absent in that region.

The transformation results into giving new information to recipient cell. As the donor *DNA* is not newly formed but is merely incorporated, it is definitely a recombination of genetic material within the cell.

(ii) TRANSFORMATION IN *Bacillus subtilis* : Certain strains of *B. subtilis* do not require tryptophan (an amino acid) for its growth as they are capable of synthesising it from other metabolites within their cells. Such strains are called tryptophan independent. Another type of strain is tryptophan dependent as they cannot grow in its absence. Such strains are incapable to synthesis tryptophan, and therefore, in culture they fail to grow unless external supply of tryptophan is met with. Both these characters are heritable.

However, tryptophan dependent strains can grow in absence of tryptophan, if *DNA* extracted and purified from tryptophan independent forms is added to its growing culture. Many cells of them turn into tryptophan independent and this progeny lasts long for future generations. Thus, it can be essentially concluded that *DNA* is the chemical substance which is carrier of genetic information.

(iii) TRANSFORMATION IN STREPTOMYCIN RESISTANT BACTERIA : Certain strains of *Pneumococcus* are streptomycin resistant and can grow in its presence. Another type of its strain is streptomycin sensitive which is killed on adding this drug in its growing culture. The traits are passed on from one cell to another during cell division in each case.

However, certain streptomycin sensitive cells can grow in presence of streptomycin, if extract obtained from streptomycin resistant strain is added to growing culture. These streptomycin sensitive cells are converted into streptomycin resistant carrying permanent hereditary changes. It was observed that in the extract of streptomycin resistant strains, *DNA* was the active component which resulted in genetic change.

The studies on transformation were extended to isotopic level using modern sophisticated techniques. Fox and Allen (1964) working with *D. pneumoniae* and Bodmer and Ganesan (1964) with *B. subtilis* and using heavy isotopes showed that segment of donor's *DNA* incorporates to recepient's *DNA* and both were covalently bound. Gurney and Fox (1968) advanced their experiments and observed that on transformation in *D. pneumoniae*, the obtained *DNA* was both genetically and physically hybrid. They showed that either chain of hybridised *DNA* could participate in the breakage and rejoining mechanism which affects transformation.

2. Transformation in Blue Green Algae

Transformation phenomenon has also been observed in certain blue green algae. H. D. Kumar (1962) while studying acquired tolerance of blue green algae to antibiotics observed that some strains of *Anacystis nidulans* were resistant either to penicillin or to streptomycin, but none to both. When both kinds of strains were thoroughly mixed and allowed to grow for two weeks, strains resistant to both antibiotics were obtained. The obtained recombination frequencies were in the ratio of 1 in 10^8 cells. Singh and Sinha (1965) also obtained this recombination property in case of *Cylindrospermum majus*. Shestakov and Khyen (1970) referred to these recombinations as an event of genetic transformation for antibiotic resistance. More recently transduction phenomenon has also been demonstrated in certain other blue green algae.

3. Bacteriophage Infection

The pattern of life cycle of a bacteriophage is yet another example for *DNA* as the genetic material. A bacteriophage consists of two parts, the head and the tail. *DNA* is found present in the head region covered by polyhedral protein coat. During infection bacteriophage becomes attached to bacterial cell and injects *DNA* into it. Protein coat remains attached outside and is thrown off after a short while. So only the *DNA* of bacteriophage enters into the host cell. It is seen after some time that large number of phage particles are produced in bacterial cell which are released after disintegration of bacterial cell.

Such an interpretation was confirmed by Hershey and Chase when they conducted the experiment using radio active isotopes. In the first experiment, the phage protein was labelled by S^{35} and was

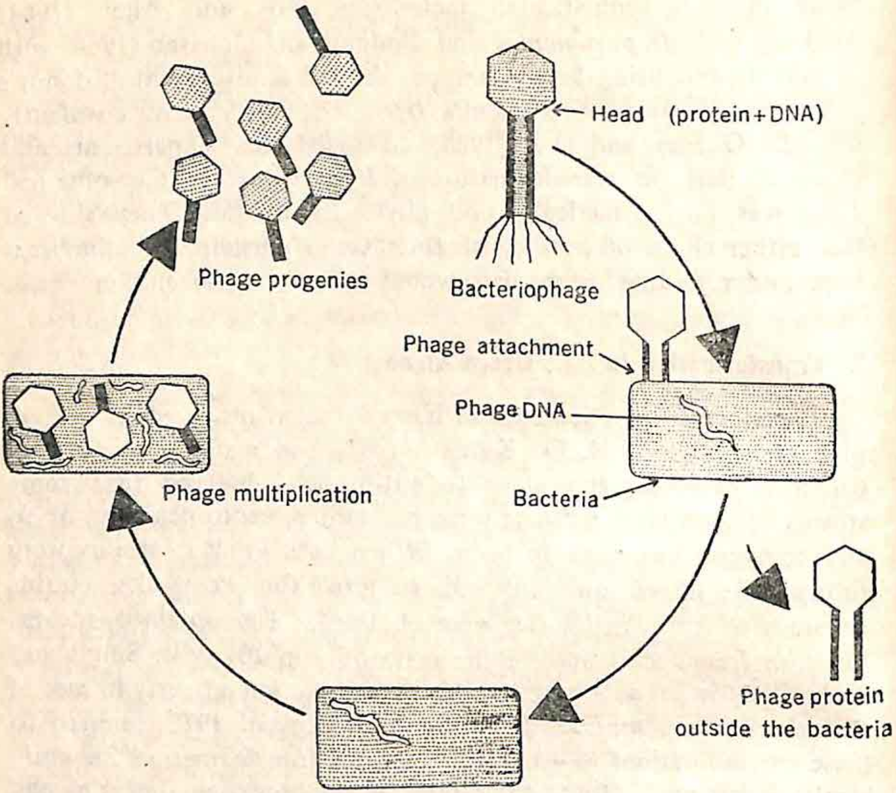


Fig. 19.2 : Life cycle of a typical bacteriophage infecting *Escherichia coli*.

allowed to infect bacteria. After the experiment it was found that S^{35} labelled virus-protein remain attached outside of the host and bacterial protein remained unlabelled. In another experiment, *DNA* of phage was labelled by P^{35} and after injection, it was seen present inside the host (bacterial cell), i.e., host contained radio active P^{35}. These two experiments further substantiate that *DNA* of phage enters into the bacterial cell and the protein coat remains outside. It also explains that *DNA* is necessary for reproduction of new virus particles or phages.

Transduction

Transduction is referred to gene transfer from one cell to another brought about by a virus. The phenomenon was first described by Lederberg, Lederberg, Zinder and Lively (1952) in *Salmonella typhimurium* in which case fragments of hereditary material may be transferred by a virus '$\varphi P22$'. In the actual process, the bacteriophage becomes associated with the *DNA* of the donor bacterium and then transfers a part of genetic material to the recipient bacterium. So the bacteriophage acts as mediator carrying the genetic material from one bacterial cell to another. Such transductions have been recorded in the case of *Salmonella* and also in *Escherichia coli.*

Among the various types of strains of auxtotrophic *Salmonella*, one requires methionine for its growth (M^-T^+) and other one threonine (M^+T^-). These two strains are selected for the experiment. A liquid culture of M^+T^- is taken, centrifuged, filtered and heated for 20-30 minutes to kill any bacterium. It is then added to culture of M^-T^+ strain. As a result of it, several prototrophic colonies (which can grow on minimal medium) appear on medium. In another case, reverse kind of experiment was performed but no phototrophs could arise. It is, therefore, concluded that M^+T^- is the donor but M^-T^+ is not the direct recipient. Other experiments show that donor bacteria are lysogens for temperate. phage φP22 which somehow transfers genetic material (*DNA*) from one host to another. The whole phenomenon is called genetic transduction.

The other salient features recorded in transduction is as follows :

(i) Transduction may be **generalised** in which a small segment from one region of bacterial chromosome can be transduced. Apparantly a short *DNA* is transduced at one time in such cases, thus quite similar to transformation in bacteria. Because these segments include several loci, genetic recombination map can be constructed from frequencies of multiple transduction. It comes almost as a single circle as in *E. coli*.

(ii) There may be a **complete transduction** in which transducing segment of *DNA* is integrated into the host's *DNA* or **abortive transduction** in which case it fails to be integrated.

(iii) Transduction may be **restricted** or **specialised** as in *E. coli* strain *K* (12)λ. It is an example of complete but restricted transduction. When λ are tested for their transduction ability, it is seen that only a few different bacterial markers are transduced, particularly adjacent to a chromosome region that controls galactose formation (Gal region).

Transduction process commences at the time of replication of bacterial *DNA* and the chromosomal loci, which are transduced, replace a part of phage chromosome. It means that transducing

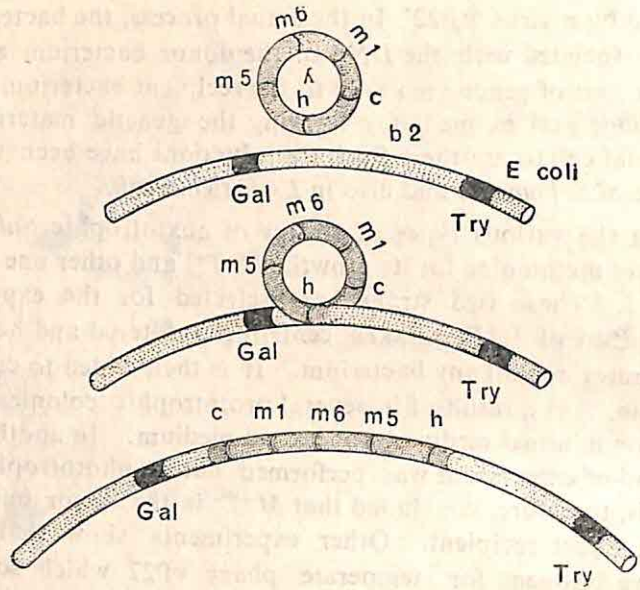


Fig. 19.3 : Diagram explaining various stages of insertion of phage *DNA* into bacterial *DNA*. Bacterial *DNA* is of *E. coli* while phage *DNA* is of lamda phage (λ). Phage *DNA* attaches to a particular site of *E. coli*. *DNA* (close to Gal region), which is soon followed by a cross over like event resulting the insertion of phage *DNA* (It may be called prophage). Jacob and Wollman referred this integrated state of pophage (Phage *DNA* + bacterial *DNA*) multiplying in synchrony with bacterial *DNA* as episome. (Diag. redrawn from Freeman, 1963)

phages are, thus, missing some of their genetic material and produce defective progenies. It has been confirmed that if *Salmonella* is infected by a single transducing *P*22 phage, lysogeny does not occur and if simultaneously infected with other non-transducing φ*P*22, lysogeny occurs. Thus, transducing phages result from the abnormal disintegration of prophage.

Infective *RNA*

In certain *DNA* organism is absent, e.g., *Tobacco mosaic virus* (*TMV*), *Poliomyeletis*, *Influenza* and *Encephalitis* viruses. In such cases another type of nucleic acid called *RNA* assumes the function of *DNA*. Such examples are not many but are limited to plant viruses which consist of exclusively *RNA* and protein. It has been now confirmed

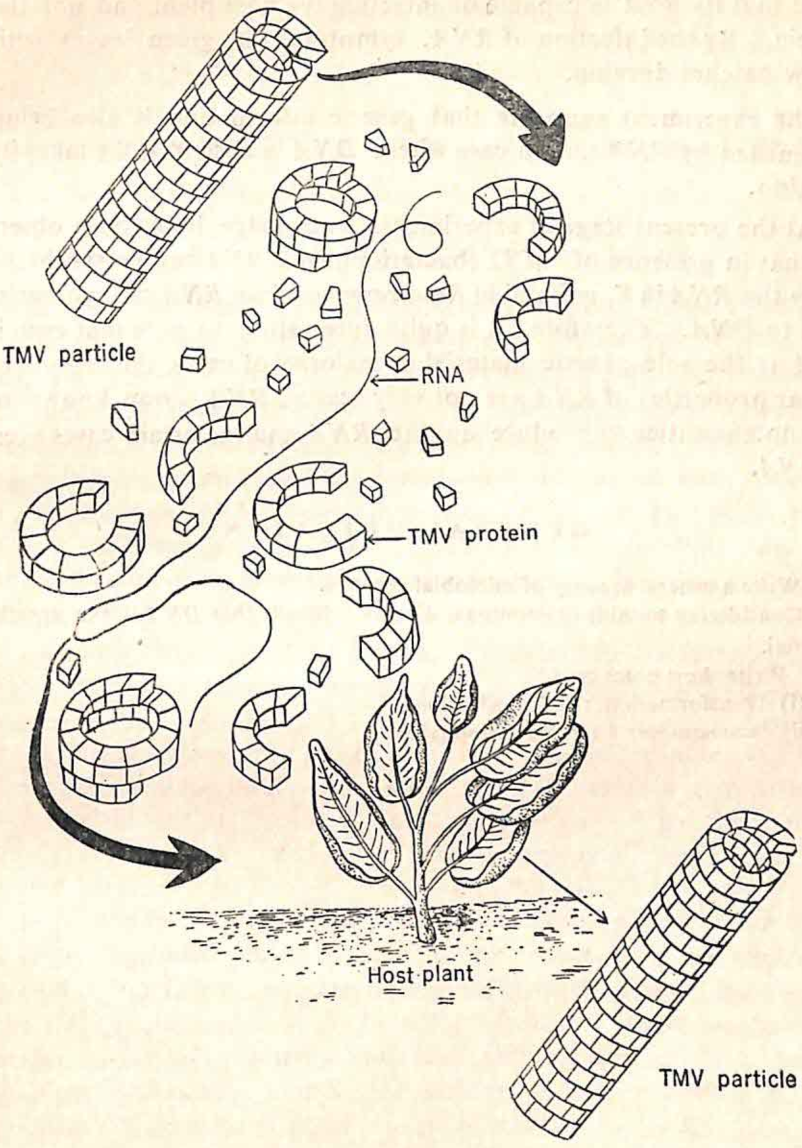


Fig. 19.4 : *TMV* particle, broken pieces showing that *RNA* has infective potentiality (See text for details).

that genetic information is being carried by *RNA* in such cases. Commonest example of this type is *TMV*.

Both *RNA* and protein of *TMV* have been chemically separated, purified and tested for their infectivity. Experimentally it has been found that its *RNA* is capable of infecting the host plant and not the protein. By the infection of *RNA*, symptoms like green leaves with yellow patches develop.

The experiment supports that genetic information is also being transmitted by *RNA* and in case where *DNA* is absent, *RNA* takes its function.

At the present stage of experimental knowledge, it has been observed that in presence of φ*MS*2 (bacteriophage), *RNA* can be synthesised by the *RNA* in *E. coli* and in *Rous cercoma virus*, *RNA* can synthesise even to *DNA*. Therefore, it is quite interesting to note that even if *DNA* is the sole genetic material in majority of cases, the reports of similar properties of *RNA* are not very scarce. *RNA* is now known to have potentialities to produce another *RNA* and in certain cases even to *DNA*.

GENERAL QUESTIONS

1. Write a general account of microbial genetics.
2. Considering suitable illustrations, discuss, critically that *DNA* is the genetic material.
3. Write short notes on :
   (i) Transformation, (ii) Transduction,
   (iii) *Pneumococcus* (iv) Bacteriophages.

■ ■ ■

CHAPTER 20

THE NUCLEIC ACIDS [DNA & RNA]

DNA (DEOXYRIBONUCLEIC ACID)

In 1869, Friedrich Meischer found in white blood cells a substance that he called nuclein. He analysed it and found that it contains 14% *N* and 2·5% *P*. It was a substance of *sui-generis*. He extended his studies and found that chief constituent of cell nucleus is nucleoprotein, i.e. a combination of nucleic acid and protein. Later it was found that the nucleic acid that occurs in combination with protein in a chromosome is the deoxyribonucleic acid (*DNA*). Since then several experiments were carried out to establish *DNA* as the chemical basis of heredity (discussed separately). It is now universally accepted.

DNA CONTENT OF NUCLEI : Boivin, Vendrely and Vendrely (1948) made successful chemical analysis of *DNA* content of nuclei of thymus, liver, pancreas and kidney of *Bos taurus* (domestic cattle). When they calculated the *DNA* content per nucleus, two remarkable discoveries were secured. First—the nuclei of various tissues have the same amount of *DNA* (6·5 pg.) (1 picogram = 10^{-12} g). Secondly—the *DNA* content of sperm nuclei was approximately half that of the diploid tissue (3·4 pg.). This led to establish that *DNA* content of non-dividing nuclei was independent of the tissues or individual and it solely depended on the chromosome content, i.e. haploid or diploid. The fact became strong argument in favour of the theory that *DNA* is the carrier of the hereditary factors. These results were further confirmed by Mirsky and Ris (1949). Besides, they found significant differences in the *DNA* content of the nuclei of different species. The amount of *DNA* in diploid tissue in *Alosa* was 2 pg. per nucleus while in *Rana* (frog) 15 pg.

DuPraw (1970) stated that "a *DNA* molecule is an exquisitely thin filament so fine that a length sufficient to reach from the earth to the sun would weigh less than half a gram. This means that an average human body contains between 10 and 20 billion miles of *DNA* double helix distributed among some trillions of cells." *DNA* in a single haploid cell of *Lilium* is about 53×10^{-12} g and if this amount is present as a single long double helix, the length of this coil would be $1{\cdot}5 \times 10^7$ microns or 15 metres and coild structure would make turns $4{\cdot}4 \times 10^9$ around the screw axis.

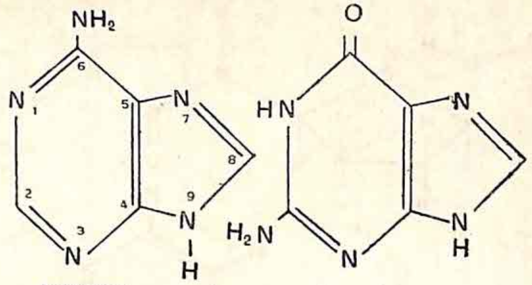
COMPONENTS OF DNA : One of the most fascinating advancement in subject of biochemistry and genetics has been the study of structure of deoxyribonucleic acid. *DNA* is a macromolecule with molecular weight ranging in millions— 6×10^6 (Sadron, 1959) and 10×10^6 (Anfinson, 1963). It is basically composed of three compounds—the 2-deoxy-D-ribose (pentose sugar), the phosphate and the nitrogen bases. The nitrogen bases belong to two heterocyclic groups—the purine and the pyrimidine. Of the purines—adenine (*A*) and guanine (*G*) and of pyrimidines—cytosine (*C*) and thymine (*T*) are found in typical *DNA*. Vischer and Chargaff (1948) by chromatographic techniques showed that the four heterocyclic bases in *DNA* are not present in equal amount. In the later studies, Chargaff (1950) drew attention to a remarkable feature that in every instance the number of molecule of adenine was almost equal to thymine and guanine equal to cytosine. The following table gives some idea of the fact.

Table. 20. I. Percentage Base composition of *DNA* of various organisms.

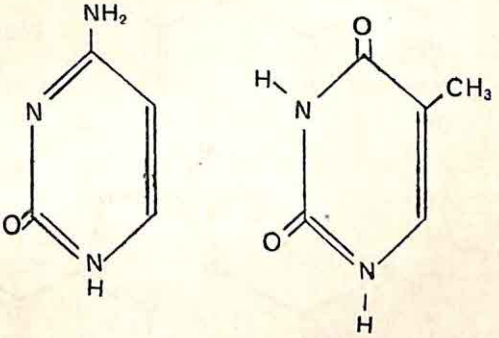
Organism	*Adenine*	*Thymine*	*Guanine*	*Cytosine*
Man (sperm)	31·0	31·5	19·1	18·4
Chicken	28·8	29·2	20·5	21·5
Sea urchin	32·8	32·1	17·7	17·7
Yeast	31·7	32·6	18·8	17·4
Escherichia coli	26·1	23·9	24·9	25·1
Vaccinia virus	29·5	29·9	20·6	20·3
Tuberculosis bacillus	15·1	14·6	34·9	35·4

Structure of DNA

BASIC STRUCTURE OF DNA : As protein consists of number of units called amino acid, *DNA* is also a polymer of nucleotides where its monomeric units are the nucleotides. Each nucleotide consists of a phosphate, a pentose sugar and an organic base. Thus, *DNA* consists of many similar units called nucleotides. The average molecular weight of the nucleotide is 330. The usual pentose of *DNA* is 2′ deoxy-D-ribose (deoxyribose) where a five membered ring is formed by four of the five carbons and a single atom of oxygen (fig. 20.13 a). The organic bases contain nitrogen and carbon atoms arranged in one or two aromatic rings (heterocyclic nitrogen bases). The organic base of a nucleotide is either a purine or a pyrimidine. The most common purines that are found in the nucleotide of *DNA* are adenine (A) and guanine (G) while pyrimidines—cytosine (C) and thymine (T).



Adenine Guanine



Cytosine Thymine

Fig. 20.1. Typical nitrogen bases found in *DNA*. Adenine and guanine belong to purine while cytosine and thymine to pyrimidine groups.

The combination of a pentose and an organic base is called nucleoside. Depending upon the type of organic base present in a particular nucleoside they are named such as deoxyadenosine, deoxyguanosine, deoxycytidine and deoxythymidine.

A nucleoside and a phosphate make up a nucleotide. The bond between *N*-base and sugar is *N*-ribosidic or glucosidic bond and that in between sugar and phosphate is ester bond. The phosphate group may be joined to either 3′ or 5′ carbon of the sugar in a nucleotide. A nucleotide in *DNA* may be called deoxyribonucleotide or deoxyribonucleoside monophosphate. The nucleotides that contain nitrogen base—*A*, *G*, *C* and *T* are called deoxyadenylic acid, deoxyguanylic acid, deoxycytidilic acid and deoxythymidilic acid respectively.

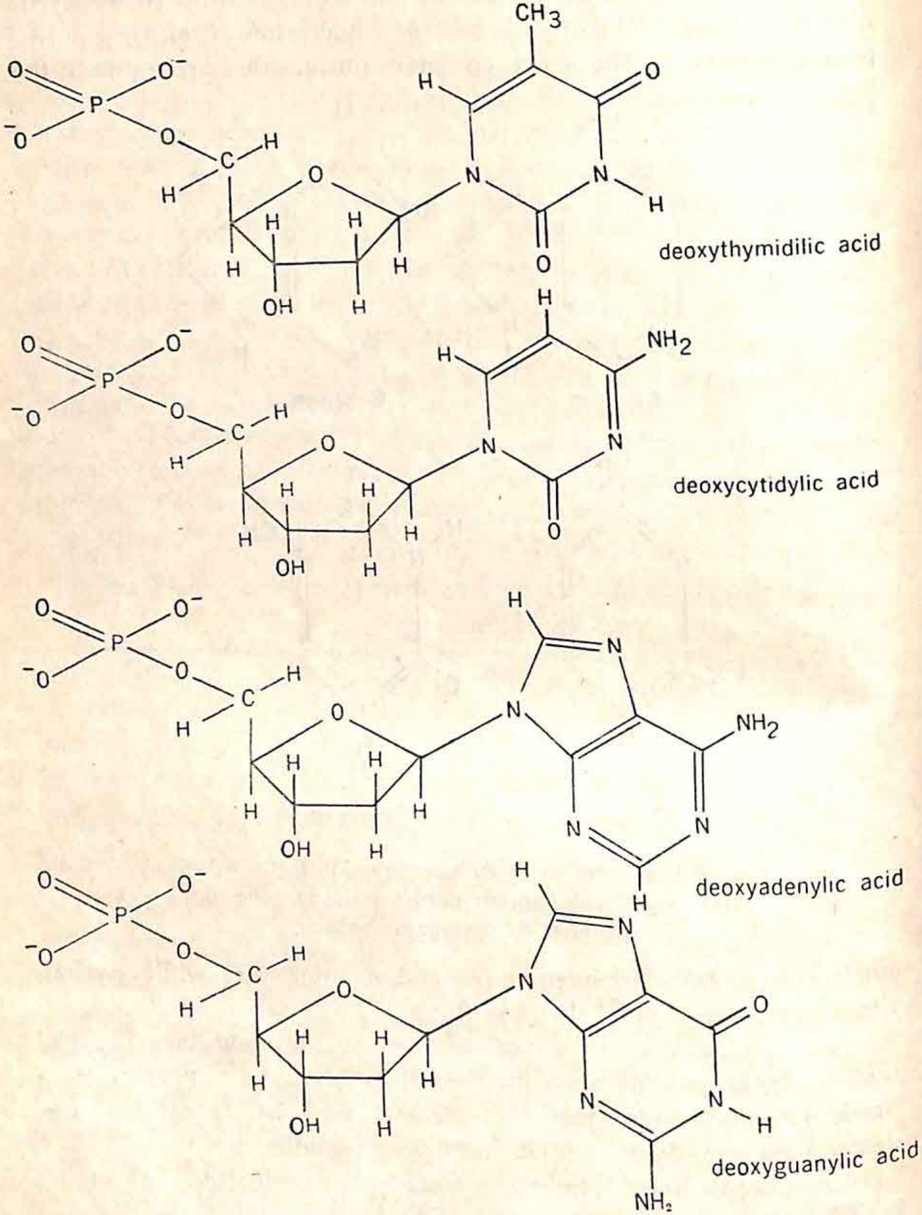


Fig. 20.2. Four types of nucleotides. A nucleotide consists of one nitrogen base, one pentose sugar molecule and one molecule of phosphoric acid. A nitrogen base together with a pentose sugar molecule is called nucleoside.

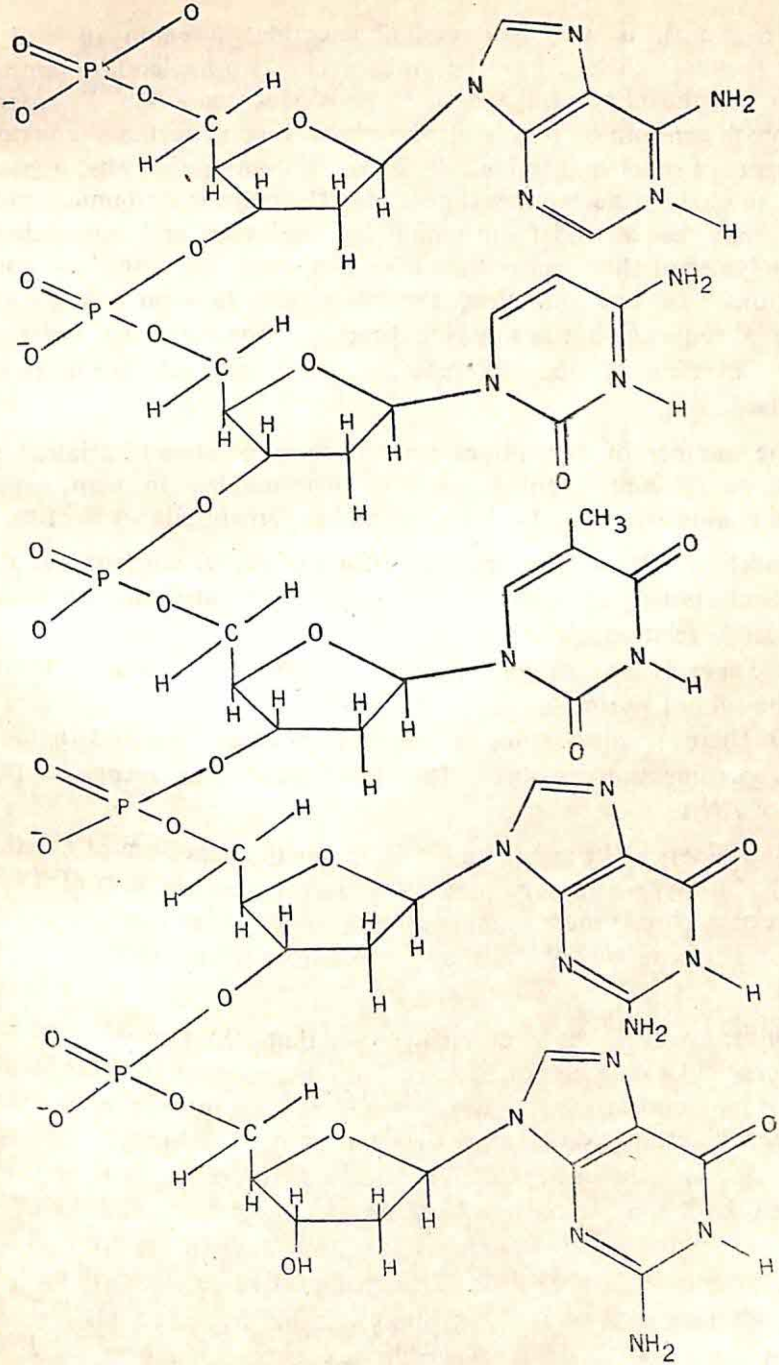


Fig. 20.3. A long polynucleotide chain. See carefully position of attachment of different nucleotides.

DNA, thus, is a polydeoxyribonucleotide, a chain in which deoxyribonucleotides form the links. The two nucleotides join by means of phosphate linkage, i. e. deoxyribonucleoside 5′ monophosphate can join by means of phosphate linkage to the 3′ position of sugar of other nucleotide (fig. 20.3). Adopting this way, a great length of chain of nucleotides is possible. The polydeoxyribonucleotide chain may be of indefinite length but is linear and unbranched. The polymer of these nucleotides does not read the same in both directions. In one direction, the phosphate to sugar linkages are in a 3′ 5′ sequence but in opposite direction, however, the order is 5′ 3′. Because of this difference, a *DNA* molecule is said to be polarised.

The number of nucleotides per chromosome shows variation as regards to different organisms such as their number in man, corn, mouse is approximately $1-1{\cdot}4\times10^{10}$ and in Drosophila $-1{\cdot}6\times10^{8}$.

Model of DNA— The arduous efforts of earlier workers and the analytical studies of many *DNA* samples revealed the following remarkable relationships.

(i) There is stoichiometric equality between the sum of purines and the sum of pyrimidines.

(ii) There is equivalence in the contents of adenine and thymine and of guanine and cytosine. This fact enabled to recognise two types of *DNA*.

(a) A T type—the sum of $A+T$ is larger than the sum of $G+C$.

(b) C G type—the sum of $C+G$ is larger than the sum of $A+T$. This relationship is more common among micro-organisms.

(iii) The content of 6-amino group equals the contents of 6-keto group.

Studies with X-ray data, suggested that *DNA* was a helical structure with a diameter of 20 A° or 2*m*μ – perpendicular to the length of the filament and with one revolution (one turn) of helix every 3·4*m*μ or 34 A°. Likewise, the titration studies indicated that the polynucleotide chains in *DNA* molecule were joined together through hydrogen bondings between the base residues. Based on various findings from analytical, electron microscopy and X-ray diffraction studies and with the consideration of contribution of earlier workers such as Wilkins, Stokes, Stahl, Meselson etc., **J. D. Watson** and **F. H. C. Crick** were able to construct a model of *DNA* which successfully solved the several problems in the field of nucleic acid chemistry.

Watson and Crick (1953a) proposed the model of *DNA* with following important characteristics.

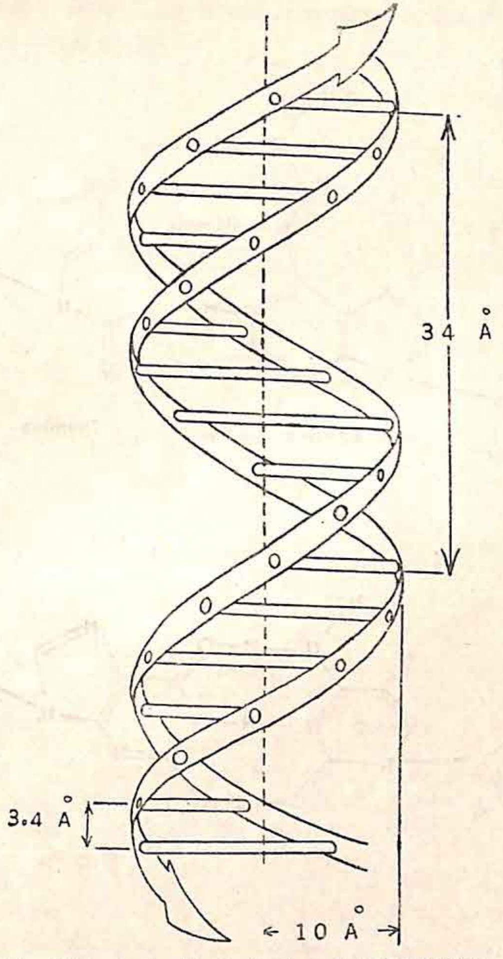


Fig. 20.4. A double helical model of *DNA* as proposed by Watson and Crick (1953a).

1. A *DNA* molecule consists of two polynucleotide chains.

2. The two chains coil in an inverted way around the assumed same axis.

3. The chains follow right handed halices, with 10 bases in each to one turn of the spiral.

4. The one chain runs inverted relative to the other, i.e. the sequence of atoms in two chains run in opposite directions.

5. The bases are present inside and phosphates on the outside of the double helix, with their planes set at right angles to the axis of helix and spaced at intervals of 0·34 mμ along it.

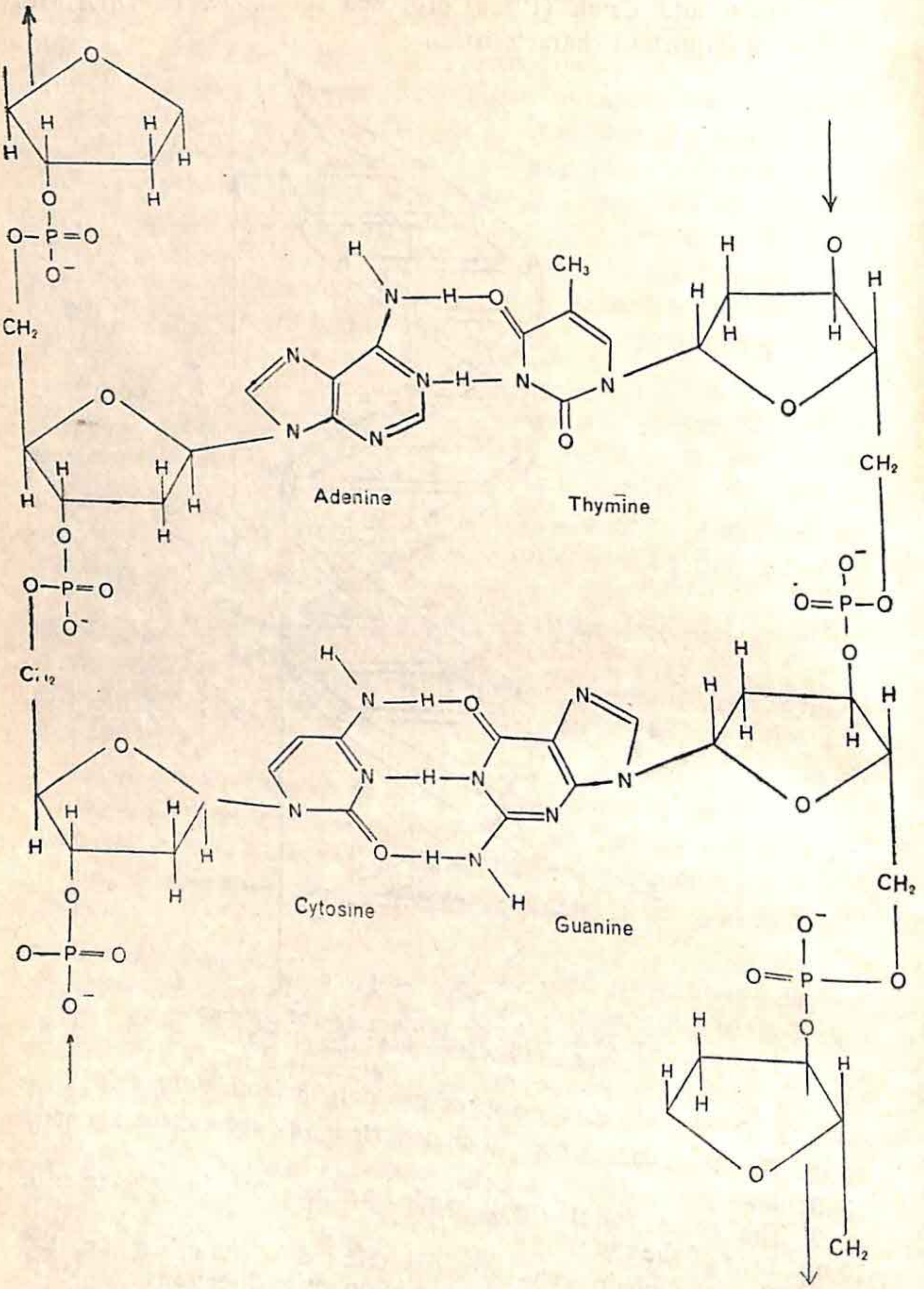


Fig. 20.5. Diagram of short section of *DNA* molecule. The opposite direction of sugar phosphate linkages in the two strands should be particularly noted.

6. The two chains of *DNA* molecule remain held together by hydrogen bondings between the bases, which join together in pairs, i.e. a single base from one chain remains hydrogen bonded to a single base from other chain.

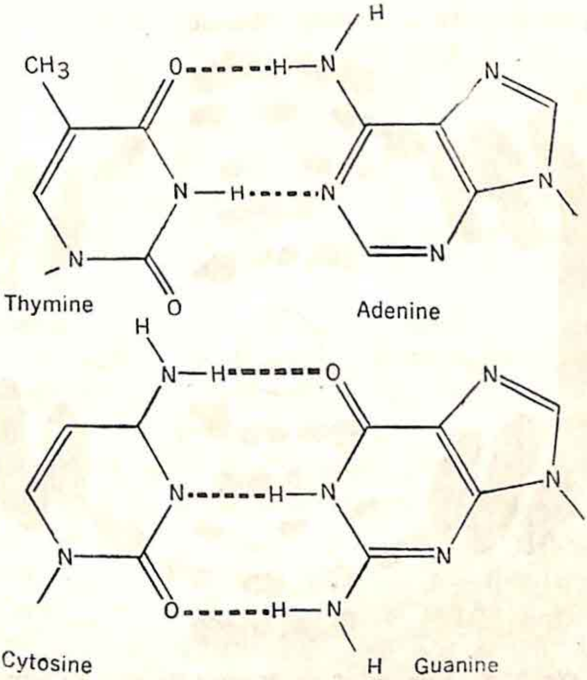


Fig. 20.6. Different nitrogen base pairs. There are three hydrogen bonds between cytosine and guanine as suggested by Pauling and Corey (1956).

7. In pairing of bases, adenine pairs with thymine and cytosine with guanine, i.e. in a pair if one base is purine adenine, the other will be pyrimidine thymine and similarly in other pair if one base is purine guanine, the other is pyrimidine cytosine.

8. There are two hydrogen bonds between each nitrogen base pair.

9. The two strands of a *DNA* molecule are complementary to one another and that the arrangement of bases on one strand fixes the arrangement on the other, i.e. if base sequence in one strand is A–G–G–T–C–A, the opposite bases on complementary strand will be T–C–C–A–G–T.

The Watson and Crick model of *DNA* obtained immediate support from the work of Wilkins, Stokes and Wilson (1953) and Franklin Gosling (1953) etc. The studies of Thomas (1956) and

Schumaker et al (1956) concluded from investigations of changes in light scattering and viscosity during partial enzymatic digestion that the *DNA* molecule must be in the form of double chains which are linked together. Meselson and Stahl (1958) using ultracentrifugal method dissolved the *DNA* of N^{15} labelled cultures of *Escherichia coli* in strong salt solution (e. g. cesium chloride) and studied its absorption of ultravoilet light which showed two bands. This gave the most impressive support to Watson and Crick model.

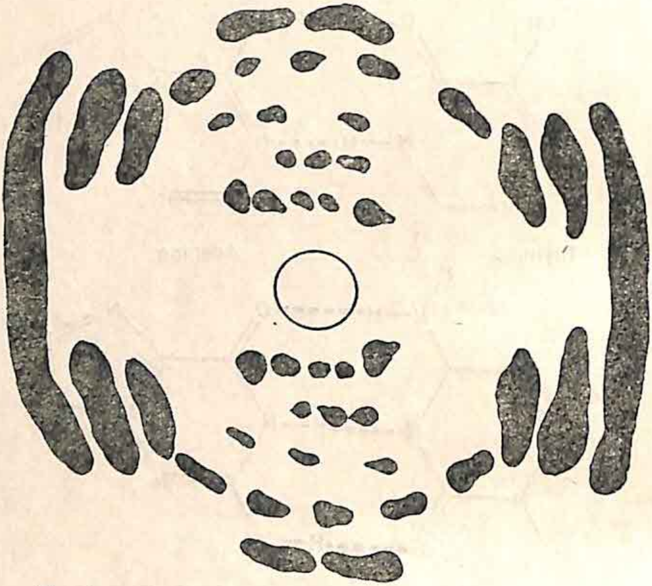


Fig. 20.7. Diagram of an X-ray diffraction pattern of *DNA* supporting double stranded model of *DNA* (From—Wilkins, 1963)

Some modifications to Watson-Crick model : Immediately after three years, Linus Pauling and Robert B. Corey (1956) proposed the presence of three hydrogen bonds between cytosine and guanine. It was a modification to Watson and Crick model but it did not influence the fundamental model as proposed by them. Commenting about the biological implications of *DNA* model, Whitehouse (1971) states that "The brilliant idea underlying the Watson and Crick model of *DNA* was that there were two complementary nucleotide chains, and that replication of the gene involved each chain acting as a template or mould for the formation of its complement. Such mechanism for copying the gene would not require gene specificity to be transferred to protein and then back to *DNA* again. Watson and Crick thus gave support to the theory that the chemical basis of heredity might be *DNA* alone." He further states that *DNA* model as proposed by Watson and Crick (1953a) was in all essential correct. But he emphasised for some modification particularly in placing the bases closer to the axis of helix. Similarly, S. R. Erlander (1970) while working on the mechanism for the synthesis of starch and its relationship to the newly proposed structural model for *DNA* proposed a model which is a hollow cylinder with the bases on the outside. It can well explain how different regulators (genetic repressor and inducers) related to this process can function. He even stated that the proofs for the Watson and Crick model such as X-rays, analyses, hypochromism and molecular dimensions are erroneous.

In Vitro **studies** : *In vitro* studies on synthesis of *DNA* molecule has been made by Arthur Kornberg who strongly supported the scheme of base pairing as suggested by Watson and Crick. The base composition of *DNA* proved quite constant within each species and insignificant differences were found between samples obtained from different organs of a given species. However, *DNA* from different species differ considerably in base composition and in their chromatographic behaviour. It has not yet been possible to unravel the sequence of purine and pyrimidines in a *DNA* chain but some indications were obtained by degradation of controlled *DNA* which reveals that these do not follow each other at random. *DNA* of different species thus differ in the proportion and in the arrangement of the bases along the common phosphate pentose backbone. It can, however, be concluded that if the genetic information is a modulation of *DNA* constitution, the arrangement of bases along the backbone must be the language in which this information is recorded.

Single stranded DNA

The data from X-ray diffraction does not necessarily reveal that *DNA* in all types of organism is universally double stranded. As usual, phages make their place unique and to this day no other organism except few bacteriophages are endowed with single stranded *DNA*. The *DNA* of mature φ*X* 174, φ*R*, *SB* and *Fd* is single stranded. Evidence for single strand comes from variety of experiment on amino group of nitrogen bases of φ*X* *DNA* which are available for reaction with formeldehyde. This is in contrast to *H*-bonded amino groups of purine and pyrimidines of double stranded *DNA*. The occurrence of single stranded *DNA* is further supported by the presence of non-equivalence of *A* and *T* and of *C* and *G* by X-ray diffraction photographs. φ*X DNA* doesn't have complementary purine and pyrimidines base composition for 24·1% of its nucleotide contain guanine, 18·5% cytosine, 24·5% adenine and 32·5% thymine. In such cases, therefore, adenine and guanine cannot be paired with thymine and cytosine. Kinetics of degradation of φ*X DNA* and that of double stranded *DNA* are also quite different. Single stranded *DNA* does not show phenomenon of helix coil transition on heating and is attacked by phosphodistase from *Escherichia coli* which acts superficially on single stranded *DNA*.

Some Unusual N-Bases of DNA

In most samples of *DNA*, four heterocyclic bases predominate. Adenine, guanine, cytosine and thymine were present in all cases but a small percentage of bases such as—5-methylcytosine (Wyatt, 1951), 6-methylaminopurine (Dunn and Smith, 1960) and 5-hydroxymethylcytosine (Loeb and Cohen, 1959) have also been reported. Both 5-methylcytosine (*MC*) and 5-hydroxymethylcytosine (*HMC*) are found in place of cytosine. *MC* is now frequently known in higher organisms such as mammals, fishes, insects and cereal grains but not in bacteria, yeast, algae and protozoans. Wyatt (1951) reported that in *DNA* of *Bos taurus* about 6% of the cytosine was 5-methylcytosine and the same about 25% in wheat. In *T*-even viruses (phages) of *Escherichia coli*, Wyatt and Cohen (1952) found that the cytosine of *DNA* was completely replaced by 5-hydroxymethylcytosine and in rare cases *HMC* also contains glucose (5-glucosyl-hydroxymethylcytosine). In *DNA* of some viruses, the usual pyrimidine is replaced by another pyrimidine, e.g. in case of φ*PBS* 1 and φ*PBS* 2, uracil occurs in place of thymine. The bovine rhinotracheitis virus contains thymine modified by substitution of a bromo group for methyl group at position 5. Likewise, *DNA* contains a variety of purines. In bacteria, upto 0·7% of all bases occur 6-methyladenine. Trace amount of

2-methyladenine, 1-methylguanine, 2-methylaminoguanine and 6-dimethylaminopurine have also been reported to occur in *DNA*.

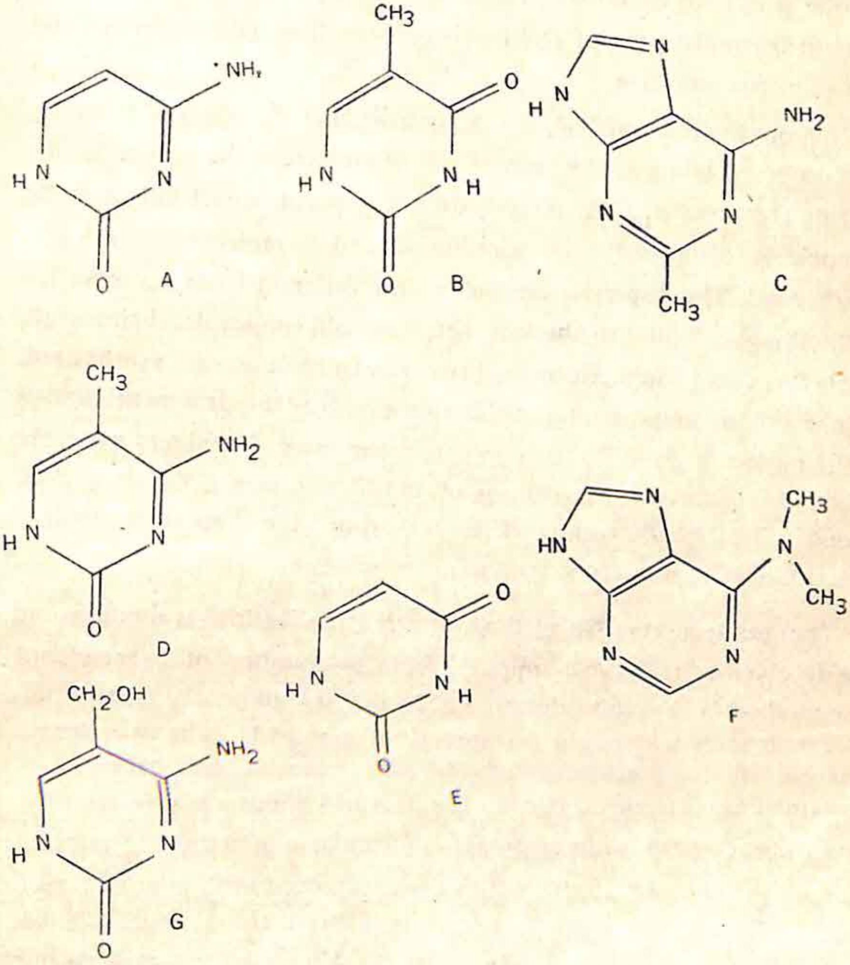


Fig. 20.8. Some usual and unusual bases of *DNA*. A—Cytosine, B—Thymine, C—2-methyladenine, D—5-methylcytosine, E—Uracil, F—6-dimethylaminopurine and G—5-hydroxymethylcytosine. (Cytosine and thymine are usual bases while others are unusual types).

REPLICATION OF DNA

Replication of *DNA* would mean the study of reproduction at molecular level. That way it has been most excitable things of the present century and nothing like biology of *DNA* has been so colourful in biological sciences. However, the process of duplication is quite simple to describe. There are three possible mechanisms to account for replication of *DNA*—(i) conservative, (ii) dispersive and (iii) semi conservative.

In **conservative method**, it is visualised that the old *DNA* strands are conserved while a new copy is synthesised from the old molecule. During the process, *DNA* strands do not separate apart but in some manner, a completely new double stranded molecule of *DNA* is synthesised. The **dispersive method** is little different from conservative method. According to this concept, the old molecule disintegrate in the process of replication and two new molecules are synthesised. The method suggests that replication involves the fragmentation of *DNA* molecule in such a way that the new daughter molecule becomes comprised of sections of an old and new *DNA* along each strand. Neither the conservative method nor the dispersive method is acceptable to modern geneticists.

The **semiconservative method** of *DNA* replication is modern and has received considerable support from a number of experiments. The method is simple to describe. In the first stage, the two strands of *DNA* molecule begin to uncoil at one end. The two strands separate by the breaking of H-bonds between the bases. The two uncoiled strands expose the naked bonds which tend to attract appropriate complementary molecular units from the pool of nucleotides. These free nucleotides, already been synthesised remain floating within the cell and during replication these approach the free ends of the original separated *DNA* strands and unite to the exposed bases. In the actual process, adenine-nucleotide combines with thymine-nucleotide while guanine-nucleotide with cytosine-nucleotide. An enzyme, *DNA polymerase*, controls the process which in addition acts as if it moves along the *DNA* strand causing the progressive separation of the two strands and an addition of a complementary one to each of these so as to complete the two halves. The new strand opposite to each original one is called

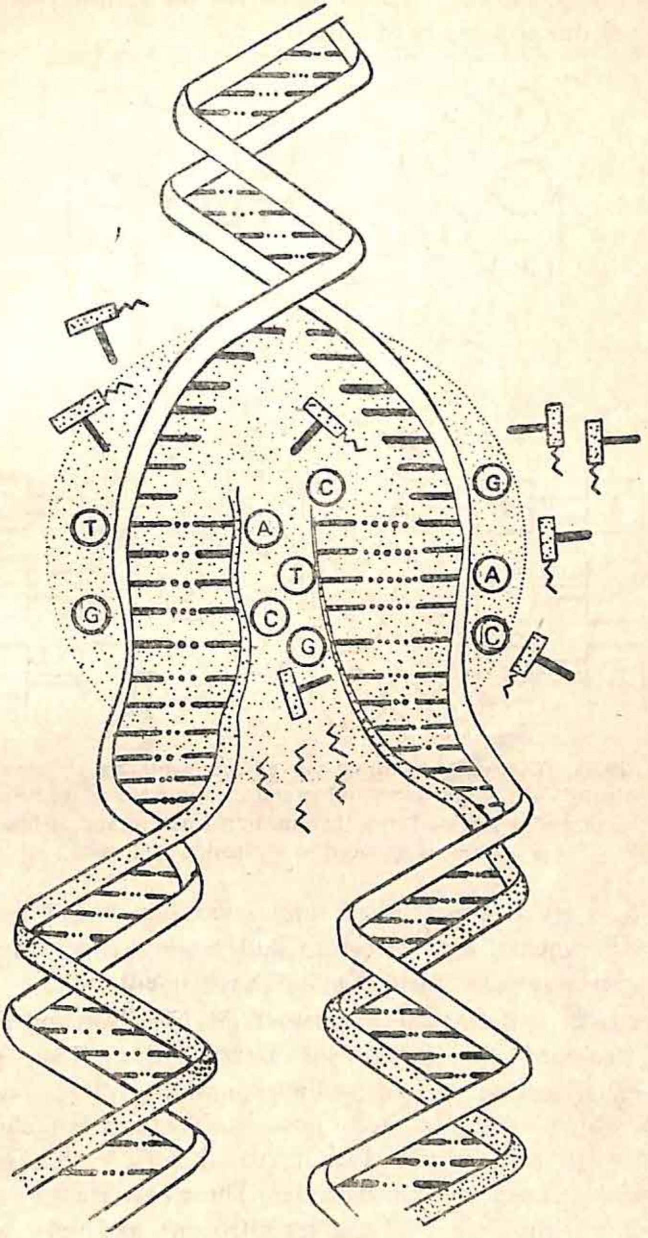


Fig. 20.9. Diagram showing semiconservative method of *DNA* replication. Replication occurs in presence of specific enzyme called *DNA* polymerase. The new strand is dotted. There are many nucleotides lying free.

replicon which is always exactly similar to the second original strand. It happens due to rigidity of base pairing.

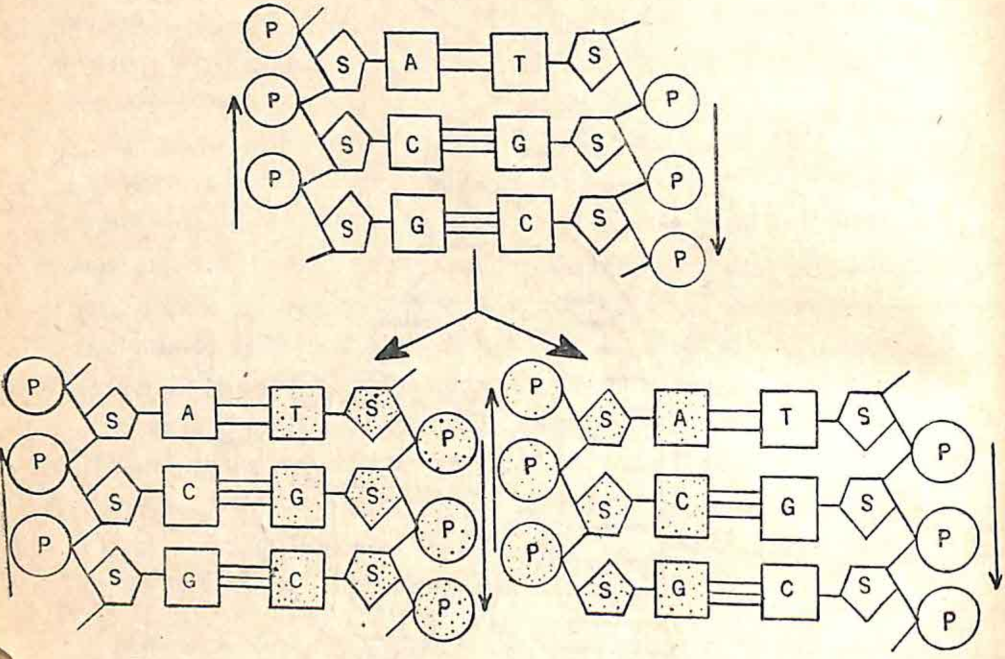


Fig. 20.10. Diagram explaining the guided synthesis of new strand (dotted) of *DNA* upon old strand. An adenine in the old strand is replaced by a thymine in the new strand, while a guanine is replaced by a cytosine and so on.

Semiconservative method of replication has been supported by many experiments, e.g. Meselson and Stahl's experiment, Cairns' autoradiography experiment, Taylor's experiment etc.

(i) MESELSON AND STAHL'S EXPERIMENT: M. Meselson and F. W. Stahl (1958) designed the experiment intelligently. They grew colon bacteria (*Escherichia coli*) on medium containing N^{15} (heavy nitrogen) due to which bacteria come to possess N^{15} atoms in their *DNA* and consequently their weight was increased. They checked up this difference by measuring their densities. These bacteria were transferred to a medium containing N^{14} (lighter nitrogen) and were allowed to grow for only one cell division. *DNA* of this generation was extracted and examined and was found that the density level now intermediate between heavy and light *DNA*. Considering semiconservative method of replication, the result is not surprising because after one division there will be fresh hybrid *DNA* molecules, each comprised of one original strand (N^{15}) and other a new strand (N^{14}). This experiment was further extended and the results of second round of cell divisions were observed. There were two bands, one at the level of N^{14} and other

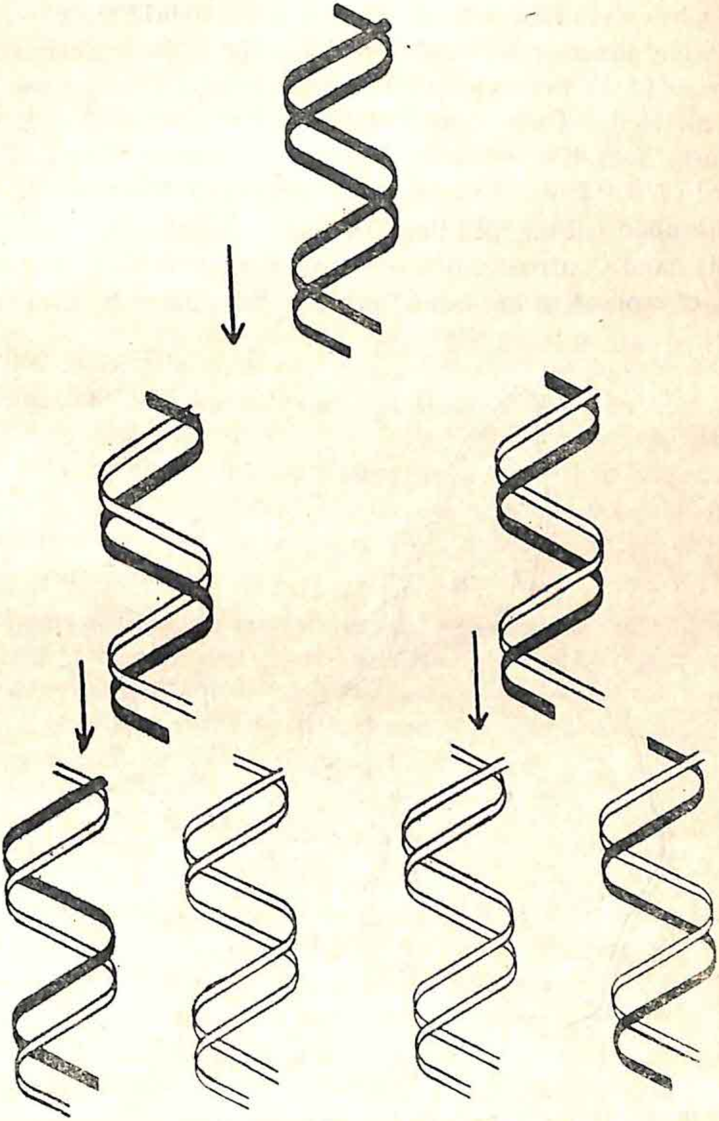


Fig. 20.11 : Diagram showing semiconservative replication of *DNA*. Parent *DNA* strands are shown black in colour. The second row shows first generation of replication and third row from the above (or bottom row) shows second generation of replication of *DNA*.

at that of hybrid (intermediate) as seen in first division. The result again suits well to the proposed model. When the results of further divisions were observed, the percentage of hybrid *DNA* decreases.

Meselson and Stahl extended yet another proof of *DNA* replication by semiconservative method. They isolated hybrid *DNA* (one strand of N^{15} and other of N^{14}) and boiled it. The hydrogen bonds between the bases of the two strands were broken and as a result two strands got separated. Their density layers were observed by centrifuging and was found two density layers, one corresponding to N^{15} and other to N^{14}. It was an additional support to the model. Incidently, it also supported the idea that *DNA* splits lengthwise.

(ii) CAIRNS' AUTORADIOGRAPHY EXPERIMENT : The semiconservative mode of replication has been further substantiated by Cairns while

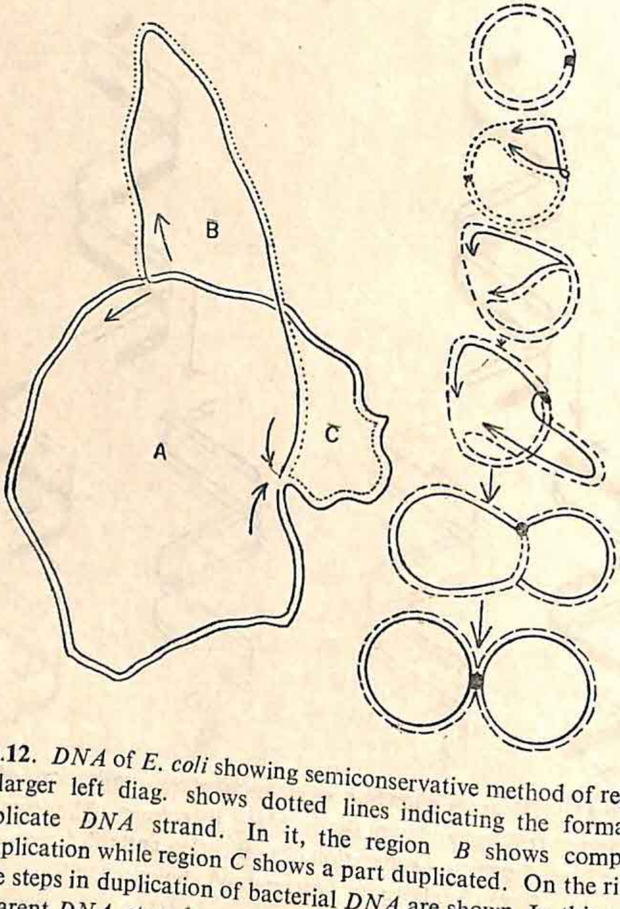


Fig. 20.12. *DNA* of *E. coli* showing semiconservative method of replication. The larger left diag. shows dotted lines indicating the formation of duplicate *DNA* strand. In it, the region *B* shows completed duplication while region *C* shows a part duplicated. On the right, the steps in duplication of bacterial *DNA* are shown. In this case, parent *DNA* strands are shown as dotted lines. (After,-Cairns.)

working on radioactively labelled 'chromosome' of *E. coli.* He selected radioactive thymidine in his studies which is found exclusively in *DNA*. (Thymine contained heavy isotope of hydrogen, H^3 to give rise to tritiated thymidine). It becomes incorporated in *DNA* and replaces ordinary thymidine. After approximately two generations, the cells were opened and their contents were carefully applied to photographic plate and stored in the dark for several weeks. When the plate was developed to reveal autoradiographic images of *DNA* molecules in various stages of duplication, the regions of presence of tritiated thymidine were observed. The autoradiographs prepared at regular intervals of replication demonstrated the semiconservative mode of *DNA* replication.

In the results, it would be speculated that the density of dots will be higher in the region where it has replicated than in the region where the replication has not taken place. The results were in conformity with the speculations. Cairns (1963) considered the lighter density dots indicate that only one of the two strands is labelled, while in heavier density dots both strands are labelled. It was actually observed in the experiment. The rate of replication could also be visualised by measuring the length of *DNA* undergoing replication in a known interval of time.

The figure that comes in an autoradiograph after second cycle of replication shows that the two arcs in the split region would never be equally labelled, e.g. one arc would be twice as heavily labelled as the other arc. This observation of Cairns clearly supported the semiconservative mode of replication.

Rate of replication

A *DNA* molecule is considerably long. About 2·2 cm. long *DNA* molecules have been isolated from human cells. DuPraw (1970) reported that *DNA* in a single haploid cell of *Lilium* is about 53×10^{-12} g and if this amount is present as a single long double helix, the length of this coil would be $1{\cdot}5 \times 10^{17}$ microns or 15 metres long. The single *DNA* molecule of *E. coli* is over a millimetre long. How replication occurs in such long molecules within few minutes is still a problem. The *DNA* of T_2-virus, composed of about 1,90,000 pairs of nucleotides requires only 20 seconds in replication, that is, 1900 nucleotides per second. Similarly, Cairns (1963) determined the rate of replication in *E. coli* and He La cells as about 20-30μ/min.

and 0·5μ/min., respectively while Taylor observed rate 2μ/min. in Chinese hamster cells. So rate may vary.

Various suggestions, a few based on experimental findings, have been put forward. A doubt about the existence of single replication site has been raised. There are suggestions that in order to hasten the process, replication may occur at both ends or at several sites simultaneously.

Favouring these aspects many suggestions are based on authentic findings. For example, in human cells if replication rate is assumed about 1 μ/min., it can synthesise about 600μ of helix during an S period of 10 hours whereas the total amount of helix to be replicated during this time is $174 \times 10^4 \mu$. It is therefore implied that the presence of at least 2900 replication sites distributed over 46 chromosomes are necessary. Each of these sites are called **replicon.** Similarly while working on mammalian cells, Huberman and Riggs (1968) observed that *DNA* molecule replicates simultaneously at several independent sites.

It is also interesting to note that not all the chromosomes replicate at a time. Comings (1967) has reported that the *X* chromosome of human females does not begin replication until 2·5 hours after the other chromosomes initiate and it continues for about 1·6 hours after most of others have finished. He further observed that chromosome 4 replicates later than chromosome 5 and number 13 later than 14 and 15.

These facts if on the one hand establish the presence of several replication sites in a *DNA* molecule, on the other these show that *DNA* replication is controlled in a reproducible manner from one chromosome to another in the same nucleus, and from place to place in the same chromosome.

RNA (RIBONUCLEIC ACID)

Like *DNA*, *RNA* also consists of repeated units called nucleotides. Most frequently it is found in the cytoplasm lying freely or associated with some organelles. The basic concept of *RNA* is similar to that of *DNA* but it differs as follows.

RNA	*DNA*
1. Pentose sugar Its D-ribose type.	Its 2-deoxy-D-ribose type in which at position 2 oxygen is absent.
2. Nitrogenous bases Adenine, guanine, cytosine and *Uracil*.	Same as in *RNA* except at the place of uracil, *thymine* is present.
3. Organisation It is single stranded and has no definite shape.	It is double stranded organised according to the model proposed by Watson and Crick (1953).
4. Place of Occurrence It is commonly found in the cytoplasm lying freely or associated with some organelles.	Its commonly found in the chromatids of nucleus.
5. The synthesis of *RNA* is dependent to *DNA*.	*DNA* is self duplicating.

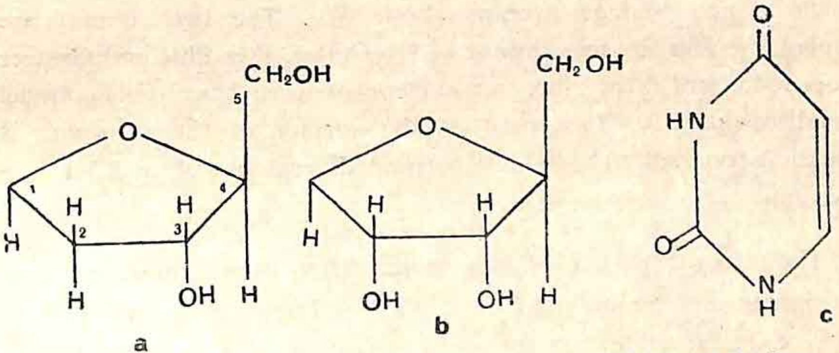


Fig. 20.13. (a) 2-deoxy—D-ribose (pentose sugar of *DNA*), (b) D-ribose (pentose sugar of *RNA*) and (c)—Uracil (a *N*-base found in *RNA*).

The recent researches in the subject have challenged the validity of these classical differences between *RNA* and *DNA*. In many cases such as φ*x* 174, φ*R*, *S* 13, *Fd* etc. (all bacterial viruses) single stranded *DNA* has been found. The double stranded *RNA* are also not unknown and have been reported in Wound tumour virus, Reovirus, Polyoma, Herpes, Vaccinia (animal viruses), T_2 bacterial virus, Turnip yellow mosaic viruses (*TYMV*) etc. In *TYMV*, branched double stranded *RNA* has been observed. In certain unusual cases, thymine in *RNA* has been recorded. Regarding the presence of *RNA* and *DNA*, many controversies have come in front. *DNA* is now known present in chloroplast, mitochondria, cytoplasm and in cell wall also (i.e. outside the nucleus). Likewise, some *RNA*s have also definite shapes. Many unusual bases have been observed in both *DNA* and *RNA*.

However, three kinds of *RNA* that take part in protein synthesis are known. These are ribosomal *RNA* (*r-RNA*), messenger *RNA* (*m-RNA*) and transfer *RNA* (*t-RNA*).

Ribosomal RNA

Found in the ribosomes of the cell, *r-RNA* constitutes upto 85-90 per cent of the total *RNA* present in an organism. The *r-RNA* combines with protein to form 30s and 50s components (sub-units) which in active stage average about 70s. The two subunits are stapled together by magnesium ions. Cotter, Mc Phie and Gratzer proposed a model for ribosome nucleoprotein structure. The model visualises that *r RNA* exposed at the surface of the ribosome is double stranded. The single stranded regions of *r RNA* are associated with internal proteins.

The exact function of ribosomal *RNA* is not known but it is considered that it should have some role related to attract and to provide the larger surface for spreading of *m RNA* over ribosomes during translation process of protein synthesis.

Messenger RNA

Constituting the small fraction (5-10%) of the total *RNA* present in the cell and carrying genetic information from *DNA*, the *m RNA*s are short-lived with a mol. wt. usually ranging from 500,000 to 2,000,000. These are synthesised in the nucleus during transcription from one strand of *DNA* in the presence of enzyme *RNA*-polymerase and are then transported through the nuclear pore to the cytoplasm where these spread on the surface of ribosomes to participate in the translation process of protein synthesis. During its synthesis from *DNA*, a hybrid molecule (*DNA-RNA*) is first formed. Obviously the so formed *m RNA* will have a complementary base sequence to one strand of *DNA*. But, which strand of *DNA* participates in transcription remains as an unsolved question. Thus, the synthesis of *m RNA* is dependent on *DNA* and since *m RNA* molecules are considerably small, a number of *m RNA* molecules can be synthesised by a *DNA* molecule.

m RNA has all other characteristics of a typical *RNA* such as presence of ribose sugar and uracil in the place of deoxyribose and thymine of *DNA*. It is single stranded and has no definite organisation. Since it brings the message of protein synthesis from *DNA*, it is called messenger *RNA*.

During its synthesis, the two strands of *DNA* split open and one of the two strands (not known which ones) begins to synthesise new *m RNA* strand. Its the common method of *m RNA* synthesis which also seeks the support of semiconservative method of *DNA* replication. Besides this normal procedure, another method has also been reported by Stern (1958) and Zubay (1962). In this method, *m RNA* synthesis by triplex formation has been suggested in which case, the two *DNA* strands do not unwind and the *m RNA* grows as third polynucleotide chain within the groove of double helix (fig. 20.14).

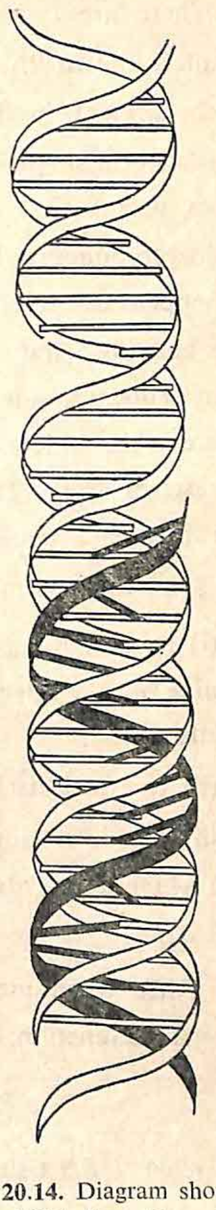


Fig. 20.14. Diagram showing *m RNA* formation by triplex formation.

Transfer RNA

There are certain general features of *t RNA* such as these are smallest known *RNA* being composed of 75-80 nucleotides and are freely present in the cytoplasm. Although like other *RNA*s, it also consists of a single strand but appears as if it is double stranded. All bases pair with corresponding bases by forming pseudotype of hydrogen bondings between the bases belonging to the same strand. It happens due to characteristic coiling. Only two parts are free from this bonding—first at terminal end where it always consists of *CCA* base sequence, adenine being always in the last and second somewhere in the middle region where three bases turn opposite making themselves free. Thus one *t RNA* differs from other ones in their free three base sequence present somewhere in the middle. A *t RNA* has got following important functions.

(i) to attach particular amino acid at the terminal end with adenine but the selection of amino acid depends on free bases of the middle region.

(ii) to attach itself at a desirous place on *m RNA*.

(iii) the liberation of amino acid in favour of other amino acid with which it gets attached with peptide bond under the instruction of *m RNA*.

s RNA when attached with amino acid is called transfer *RNA* to refer its function, i.e.

$$s\ RNA + \text{amino acid} \longrightarrow t\ RNA$$

While *t RNA* shows some remarkable features, it has become of paramount importance for understanding the mechanism of translation during protein synthesis. Scientists persued the study on its various aspects such as on determination of structure and sequence of nitrogen bases and its mode of action. Holley et al (1965) for the first time determined the nucleotide sequence of transfer *RNA* molecules of *Saccharomyces cerevisiae* and *Escherichia coli*.

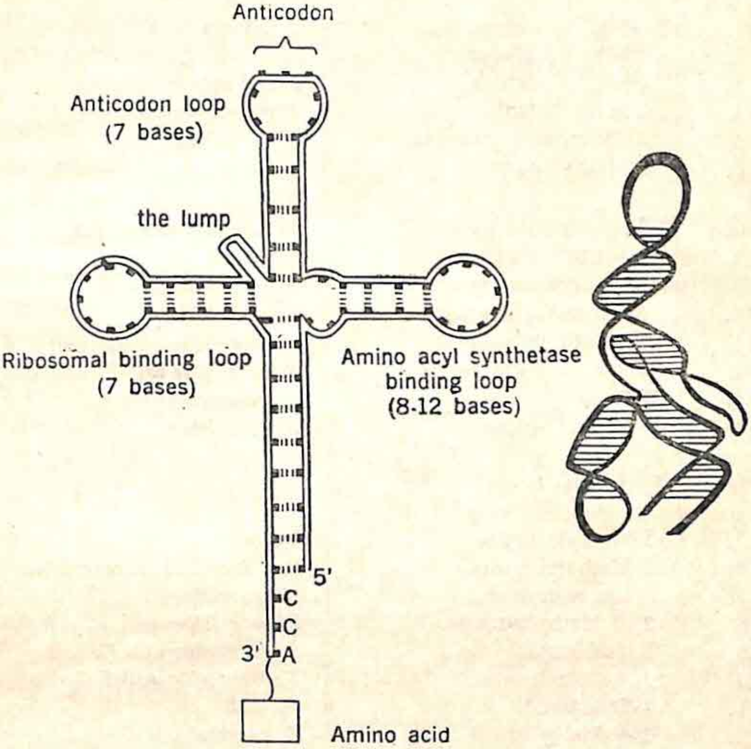


Fig. 20.15. Proposed clover leaf model of *t RNA* (on the left) and a modified diagram showing folded clover leaf model (on the right).

The *t RNA* molecule for alanine in *S. cerevisiae* was found to consist of a chain of 77 nucleotides showing a possible configuration as a clover leaf shape (fig. 20.15). They found that it has normally four arms (3 leaflets and a stalk to the clover leaf) but the fifth arm may be present. One of the most important points to keep in mind is that *t RNA*s have been found to contain several unusual bases. These are listed as below.

Table 20. II : *Showing presence of unusual bases in different t RNAs.*

S. No.	*Name of the unusual base*	*Organism in which their presence is reported*
	Purine Bases	
1.	1-Methyladenine	*S. cerevisiae*, Pig liver.
2.	N^6-Dimethyladenine	,, ,,
3.	2-Methyladenine	*S*-180 ascites
4.	6-Methyladenine	Rat liver
5.	N^6-Isopentenyladenine	*S. cerevisiae*
6.	1-Methylguanine	*S. cerevisiae*, Rat and Rabbit liver
7.	2-Methylguanine	Mouse adenosercoma
8.	7-Methylguanine	Pig liver
9.	N^2-Methylguanine	*S. cerevisiae*
10.	N^2-Dimethylguanine	*S. cerevisiae*
11.	N^7-Methylguanine	*S. cerevisiae* and *E. coli*
12.	2′-0-Methylguanine	*S. cerevisiae* and *E. coli*
13.	Inosine	*S. cerevisiae*
14.	1-Methylinosine	*S. cerevisiae*
	Pyrimidine Bases	
15.	3-Methylcytosine	Yeast
16.	5-Methylcytosine	Rat liver and *S. cerevisiae*
17.	N^6-Acetylcytosine	*S. cerevisiae*
18.	2′-0-Methylcytosine	*S. cerevisiae* and *E. coli*
19.	Ribothymine	*S. cerevisiae* and *E. coli*
20.	5, 6-Dihydrouracil	*S. cerevisiae* and *E. coli*
21.	4-Thiouracil	*E. coli*
22.	2′-0-Methyluracil	*S. cerevisiae*
23.	Pseudouracil	*S. cerevisiae* and *E. coli*

These unusual bases occur chiefly in the unpaired regions of *t RNA* and have not been found near the placement of attachment of amino acid. Their definite position in different *t RNA*s of various organisms suggest that these particular nucleotides may have persisted unchanged in position over the immense period of evolution.

In general, the newly proposed clover leaf model of *t RNA* has the amino acid arm, the anticodon arm and the thymine arm with an extra arm in certain cases. The function of the amino acid arm is the attachment of specific amino acid while that of anticodon arm is to register with the codon of the *messenger RNA*. The recognition site for the specific enzyme that catalyses the attachment of amino acid is not yet known.

Lake and Beeman (1968) found that an open clover leaf model is quite unsatisfactory. They proposed a folded clover leaf model in which the helical regions were fitted together as closely as possible and the arms lay in two pairs, the amino acid and anticodon arms pointing in one direction and the guanine and thymine arms in the opposite direction with their loops close together (Fig. 20.15).

GENERAL QUESTIONS

1. Write an essay on structure and composition of *DNA*.

2. Discuss critically the structure of Watson and Crick model of *DNA* in light of recent researches.

3. How will you discuss that modern researches are posing threats to Watson-Crick model of *DNA* ?

4. Discuss critically the various types of *RNA*s found in the cell. How are they different from each other ?

5. How would you account for semi-conservative method of *DNA* replication ?

6. Write short notes on— (i) Polynucleotides, (ii) Some unusual bases of *DNA*, (iii) *t RNA*, (iv) Replication of *DNA* and (v) Watson and Crick.

■ ■ ■

CHAPTER 21

BIOSYNTHESIS OF PROTEINS

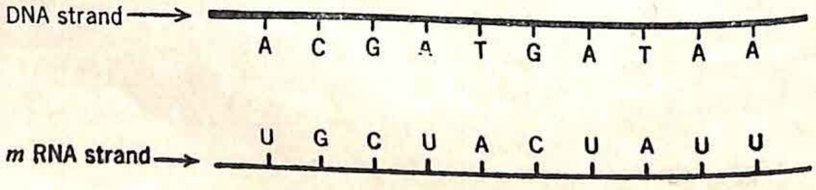
DNA controls the sole biometabolic activities of the cell or organism by controlling and directing the enzyme synthesis which are as such essential for driving the various metabolic reactions. This concept is also known as **central dogma.** So *DNA* controls the nature and specificity of enzymes. Enzymes, on the other hand, consists of proteins which are polymerised form of unit structure called amino acid. However, only twenty types of amino acids are known to participate in the composition of proteins while in each protein molecule, several hundreds amino acid molecules are arranged in orderely sequence.

So in true sense, the problem centres around the mechanism that how *DNA* molecules execute the process of selection of amino acids and get them organised into a definite number and position. It has been proved that *DNA* via *m RNA* (*messenger RNA*) has potentiality to select particular amino acids and get them tagged into the synthesising protein molecule. The information for selecting specific amino acid out of twenty, is found in the base sequence of *DNA* molecule.

For the sake of convenience, the mechanism involved in the biosynthesis of protein can be divided into two main stages.

1. Transcription (Formation of *m* RNA)

The portion of *DNA* destined to synthesise one protein molecule (or enzyme) unwinds and the two strands split apart. One of the two strands begins to choose complementary nucleotides present in its surrounding, i.e. if on that *DNA* strand, the base is cytosine, it will attach only guanine nucleotide and no one else in the new strand. The whole process runs in the presence of *RNA* polymerase enzyme.



The process of formation of long complementary strand of nucleotides continues till it is completed. When a new strand is formed, it contains opposite (or complementary) base sequence of parent *DNA* strand. The newly formed strand is called *m RNA* since it carries the message of *DNA*. The whole process is called transcription as *DNA* base sequence is transcribed into *m RNA* base sequence. (The details of *m RNA* are given in the Chapter 20.)

$$\text{DNA} \xrightarrow[\textit{RNA polymerase}]{\text{Transcription}} m\ \text{RNA}$$

The *m* RNA now comes out of the nucleus in the cytoplasm and attaches itself on the smaller subunit of ribosomes which are found attached on the surface of endoplasmic reticulum. It has been proposed that *r RNA* attracts *m RNA* and provides larger surface to spread. At a time, one *m RNA* may spread on more than one ribosome (upto 4 or 5). The whole complex (ribosomes + *m RNA*) is called polysome or polyribosome. The *m RNA*'s attachment on ribosome is so as to keep the bases free for their further participation in the process.

2. Translation

Before understanding the actual process of translation, the structural details of *t RNA* (*tranfer RNA*) is necessary to make clear.

TRANSFER RNA : There is yet another type of peculiar *RNA* found freely present in the cytoplasm called *soluble RNA* (*s RNA*). Although, like other *RNA*s, it also consists of single strand but because it is so coiled that it may appear double stranded (the details are given in the Chapter 20). *s RNA* when attached with an amino acid is called *t RNA* (*transfer RNA*).

$$s\ RNA + \text{amino acid} \rightarrow t\ RNA$$

AMINO ACID ACTIVATION : Before an amino acid gets attached to adenine of *s RNA*, it is first to be activated. The activation is carried out by the *ATP* molecule. In the first step, amino acid molecule is activated by the reaction with *ATP* in the presence of specific enzyme called amino acyl synthetase. Magnesium is also

$$\boxed{\text{Amino acid} + \text{Enzyme} + \text{ATP} \longrightarrow \text{Amino acid}-\text{AMP}-\text{Enzyme} + \text{PP}}$$

involved in this reaction. In the process, amino acid adenylate is formed and pyrophosphate is released. *AMP* joins the amino acid by a high energy bond forming an enzyme bound-amino acid-*AMP* complex.

This complex combines with *s RNA* which is specific for the

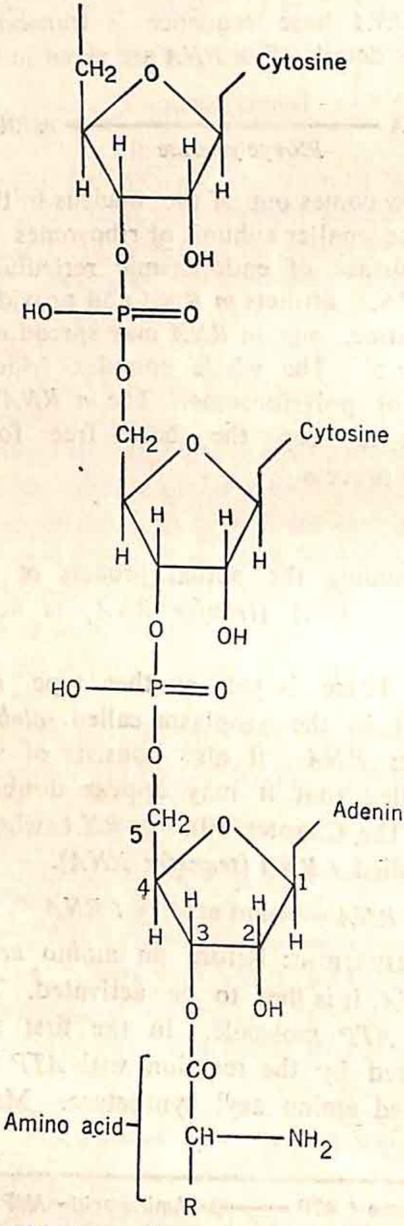


Fig. 21.1. The place of amino acid attachment in *t RNA*. The diagram shows terminal end of *t RNA* (*CCA*) with an attached amino acid molecule at position 3 of ribose sugar molecule.

amino acid. The amino acid is always attached at *CCA* end of *s RNA*. The actual place of its attachment is the 2′ position of ribose sugar of adenine nucleotide (Fig. 21.1).

s RNA when associated with an amino acid is called *t RNA*. This *t RNA*—amino acid remains in active form.

$$\boxed{\text{Amino acid} - \text{AMP} - \text{Enzyme} + s\,\text{RNA} \longrightarrow \text{Amino acid} - t\,\text{RNA} + \text{Enzyme} + \text{AMP}}$$

TRANSLATION : It can be better explained by dividing it into two different steps.

(A) *Composite behaviour of ribosomes* : Ribosomes are the sites of protein synthesis translation process. In the cytoplasm, these are found dissociated into their sub-units (30s and 50s) when not participating in protein synthesis. The *m RNA* attaches to its 30s subunit through its 5′ end of *m RNA* carrying *AUG* codon (almost every *m RNA* carries first codon *AUG*, rarely *GUC*). This attachment is carried out in presence of protein factor (F_3).

$$\boxed{30\text{s} + m\,\text{RNA} \xrightarrow{F_3} 30\text{s} - m\,\text{RNA}}$$

First *t RNA* carrying methionine called *f*-met-*t* RNA_f^{met} attaches with 30s – *m RNA* complex. It is facilitated by protein factor (F_1).

$$\boxed{30s - m\ RNA + \text{f-met} - t\ RNA^{met}_{f} \xrightarrow{F_1} 30s - m\ RNA\text{-}\ \text{f-met} - t\ RNA^{met}_{f}}$$

Electron Microphotographs

Plate 21. I. Polysomes from rabbit reticulocytes, shadowed with platinum (Courtesy of Professor A. Rich.).

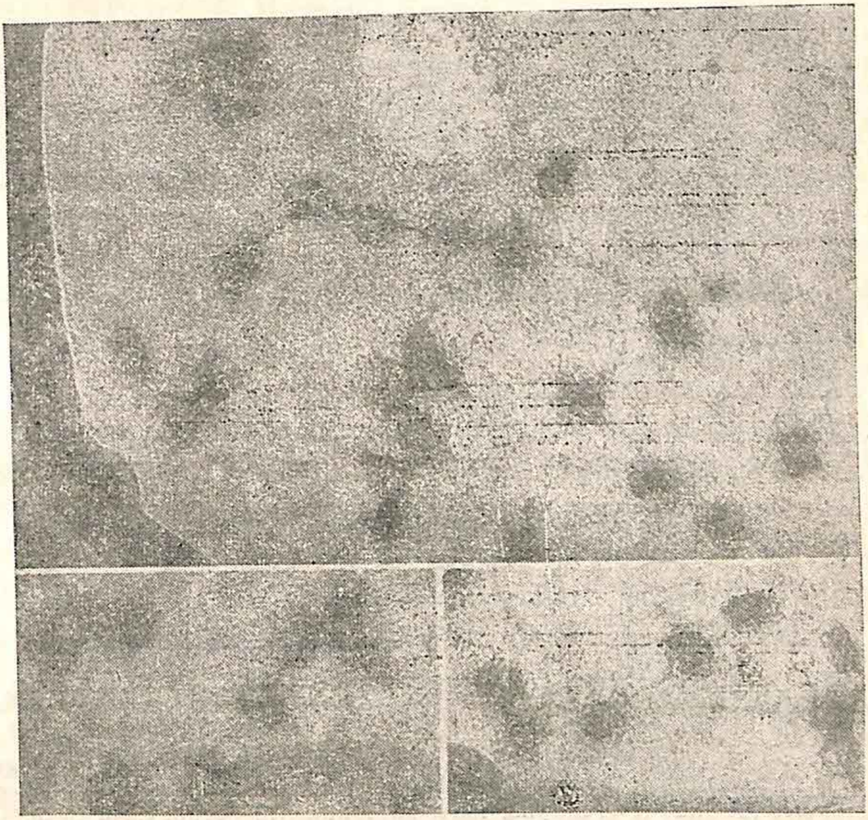


Plate 21. II. Electron micrographs of reticulocyte polyribosomes stained with uranyl acetate (Courtesy of Professor A. Rich)

At this stage 50s subunit of the ribosome associates with its 30s subunit and forms 70s ribosome. The process occurs in presence of protein factor (F_2).

$$\boxed{30s-m\,RNA\text{-f-met-}t\,RNA_f^{met} + 50s \xrightarrow{F_2} 70s-m\,RNA\text{-f-met-}t\,RNA_f^{met}}$$

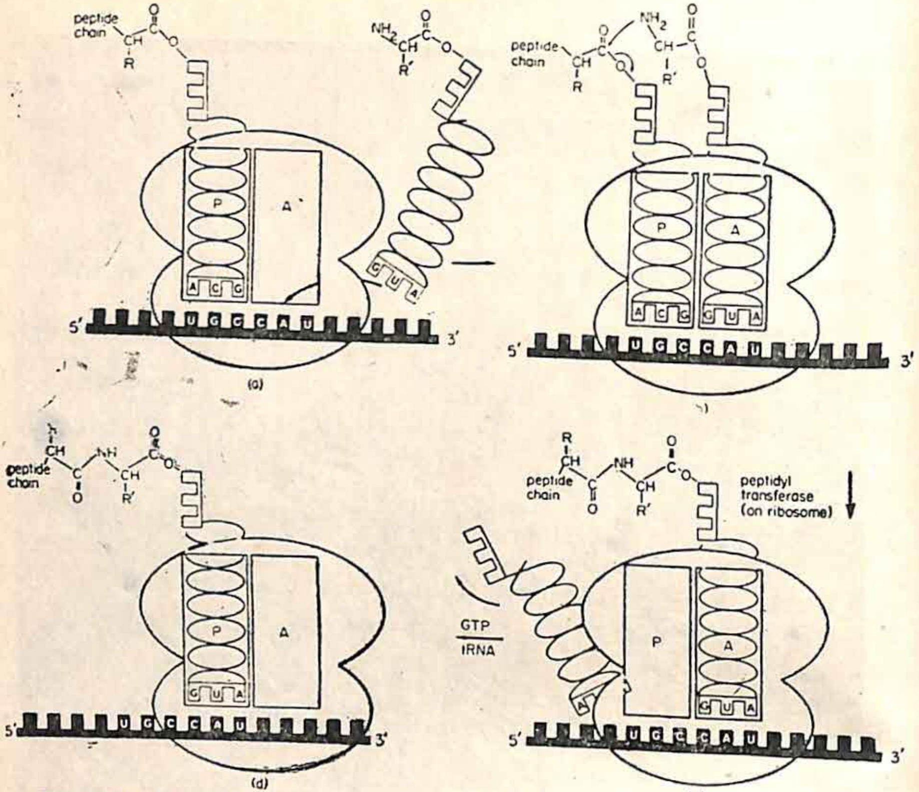


Fig. 21.2. Diagram showing ribosome cycle of peptide bond synthesis. (a)—a ribosome with *t RNA* carrying the so far synthesised peptide chain in the *P* site. The other *t RNA* is entering the *A* site of the ribosome, (b)—the two *t RNAs* while present on *P* and *A* sites show a new peptide bond synthesis which is accomplished by transfer of the polypeptide chain attached to the *t RNA* in the *P* site to the amino-acyl—*t RNA* in the *A* site. It is believed that this reaction takes place in presence of an enzyme bound to the 50S subunit of ribosome, (c)—the *t RNA* in the *P* site is released which leaves polypeptide chain attached to the *t RNA* in the *A* site, (d)—the ribosome moves one triplet to the right in a step requiring GTP hydrolysis. This, however, restores state (a) but now polypeptide chain contains one more amino acid molecule.

(Adopted from B. M. Lewin, 1970)

Once this complex is formed, elongation of polypeptide chain begins which involves three important steps—(i) binding of *AA-t RNA* (amino-acyl complex) at the *A* site of ribosome; (ii) formation of peptide bond and (iii) translation of peptidyl *t RNA* from *A* to *P* site.

Each ribosome has two cavities–*P* (polypeptidyl site) and *A* (amino-acyl site) sites. *AA t RNA* joins either *A* or *P* site (not definitely known) but ultimately it has to be present on *P* site to make *A* site available for next *AA t RNA*. While *AA t RNA* enters *A* site, a molecule of *GTP* (guanosine triphosphate) and *TF* I (transfer factor I) are required. *GTP* is released as *GDP* and a phosphate group with *TF* I. The liberated energy is utilised (its mechanism is not precisely known).

The formation of peptide bond occurs when free carboxyl group $(-COOH)$ of peptidyl *t RNA* is present at *P* site and free amino $(-NH_2)$ with *AA t RNA* at *A* site. Later *AA t RNA* present on *A* site translocates to *P* site in presence of *GTP* and *TF* II (transfer factor II). During the whole process, a relative movement of ribosome and *m RNA* occurs in order to make available next codon at *A* site (reading off the codon). How this relative movement occurs is not adequately known. Once the protein synthesis is completed, the two subunits of ribosome dissociate (i.e., $70s \longrightarrow 30s+50s$) again.

(B) *Formation of polypeptide chain* : A particular type of *t RNA* (which has complementary base triplet to *m RNA*) with its specific amino acid is attracted by the particular site of *m RNA* where it pairs with *m RNA* codon. The pairing of *t RNA* takes place with corresponding base triplet of *m RNA*. The triplet present in *m RNA* is called codon and that in *t RNA* is anticodon. They are complimentary to each other. Simultaneously, the second *t RNA* comes to join the second site of *m RNA*. As a result, amino acid of first *t RNA* is attached with amino acid of second *t RNA* by forming a peptide bond.

$$\text{amino acid}_1 + \text{amino acid}_2 \longrightarrow \text{amino acid}_1 - \text{amino acid}_2$$

As soon as the first *t RNA* loses its amino acid, it is made free in the cytoplasm. The whole process of pairing of *m RNA* with *t RNA* with specific amino acid is called translation. In translation, thus the *t RNA* anticodon base sequence will be similar to that part of *DNA* base sequence. That's why the process has been called translation. These steps continue till the final steps are completed.

A final product is a long polypeptide chain which is a major structural unit of protein or enzyme molecule.

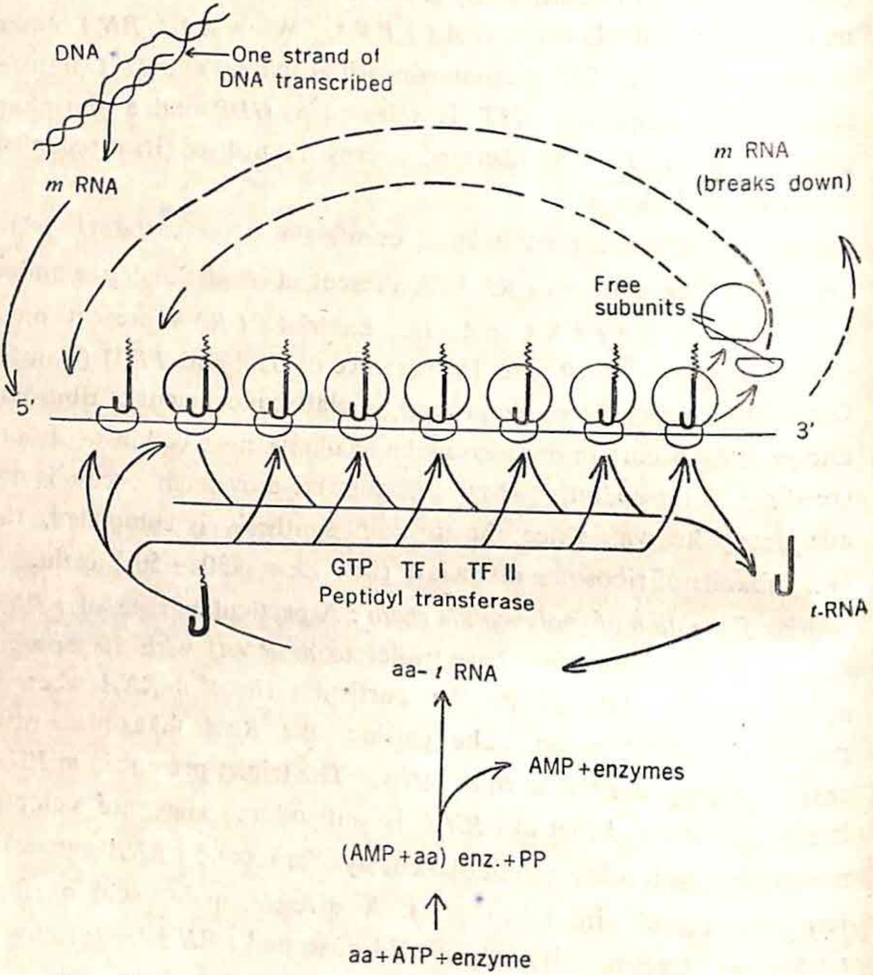


Fig. 21.3. Diagram explaining a summary of various steps of formation of a polyribosome and protein synthesis.

So the protein synthesis occurs in two steps and involves *DNA*, *m RNA*, *s RNA* and amino acids. Transcription process occurs inside the nucleus and translation in the cytoplasm.

$$\text{DNA} \xrightarrow{\text{Transcription}} m\,\text{RNA} \xrightarrow{\text{Translation}} t\,\text{RNA}$$

$$\longleftarrow \text{- - - - - Nucleus - - - - - -} \rightarrow\leftarrow \text{- - - - - Cytoplasm - - - - - -} \longrightarrow$$

(The whole concept is known as Central dogma.)

Thus, one *m RNA* is capable of producing one particular protein (enzyme) and since it is a unit of function and dependent of *DNA* it is considered to be equivalent to a gene present in a *DNA* molecule. The whole process occurs strictly in accordance with the base sequence present in *DNA*. In the process, *DNA* acts like a king, *m RNA* like minister, *s RNA* like soldiers and polysome like scrutinising officers selecting public representatives (amino acids) to form an army (protein molecule) in order to perform one function (function of protein as enzyme).

GENERAL QUESTIONS

1. Write an essay on protein synthesis.
2. Discuss critically the role of ribosomes during protein synthesis.

■ ■ ■

CHAPTER 22

THE GENETIC CODE

Genetic Code

While the subject genetic code is of utmost importance, it is not only bit technical but also quite interesting. It is by far the most modern and calculative chapter of molecular genetics. Many facts in the subject have been interpreted on the basis of mere calculations. The genetic code answers to a question that how four types of nucleotides present in *m RNA* serve to direct the synthesis of protein by the assembly of specific amino acids in a specific sequence and also in the definite numbers. Of the twenty amino acids so far known to participate in protein synthesis, there is a specific selection for each amino acid which takes place by the involvement of only four types of *m RNA* nucleotides. If presumed that one nucleotide would direct for one amino acid selection or one nucleotide would code for one amino acid, there could only be four codons and the four nucleotides of *m RNA* would be enough for just four amino acids. It is therefore imperative that more than one nucleotide of *m RNA* should have to be used for coding one amino acid. How many should then be used ? If two, then only sixteen duplets are possible which again would not be enough to code for all amino acids. Thus it may be concluded that a minimum of triplet code would be required. So it was just a theoretical calculation that three nucleotides together (triplet) are involved for coding each amino acid and there would be therefore sixty four triplets in all. Triplets may also be called codons. Later, this theoretical presumption became true. The existence of triplet codons have been well substantiated experimentally by the arduous efforts of Nirenberg, Leder, Ochoa, Khorana etc.

Nirenberg and his associates while working on *in vitro* studies on *Escherichia coli* enjoyed the credit of deciphering of genetic code. Nirenberg used enzyme extracts of *RNAase* and synthesised a polynucleotide chain containing one kind of base, i.e., polyuridylic chain. To this poly-*U* chain, he added active cell free systems (*t RNAs*) and labelled mixture of twenty amino acids separately and individually. He found the formation of polypeptide chain which contained only one animo acid—phenylalanine (polyphenylalanine). An immediate conclusion from the experiment was drawn that the codon for amino acid phenylalanine was a sequence of three uracil nucleotides (*UUU*) in *m RNA*. Similarly he synthesised other poly-*G*, poly-*C* and poly-*A* nucleotides and employing the same technique he concluded that base triplets *GGG*, *CCC* and *AAA* code for glycine, proline and lysine, respectively.

Further problems for the synthesis of polynucleotides containing other possible combinations of triplets were difficult. Khorana quickly devised an ingenious technique to resolve them. He used synthetic polydeoxyribonucleotides (synthetic *DNA*) and synthesised polyribonucleotides (*m RNA*) of known sequence for directing cell free synthesis of polypeptides. The amino acid sequence of these polypeptides was later determined. Say for example (in highly simplified form) if *C* and *U* (two bases) were taken, these would be repeatedly present throughout the length in a form *CUCUCUCUCU-CUCUCU* and it would produce two types of triplets, i.e., *CUC* and *UCU* (only possibility) and if three bases—*A*, *C* and *U* were taken, a polynucleotide would be *ACUACUACUACUACU*, i.e., triplets may be *ACU*, *CUA*, *UAC*. During experimentation he found that if two bases *C* and *U* were taken in repeating sequence, there was formation of polypeptide chain composed of leucine and serine and if three bases *A*, *C* and *U* were taken, a polypeptide chain composed of threonine, arginine and glutamic acid was formed. He immediately concluded that the codon for leucine is *CUC* while for serine, threonine, arginine and glutamic acid are *UCU*, *ACG*, *CGA* and *GAC*, respectively. He further elaborated his experimental technique and discovered about 25 codons—coding different amino acids.

On the basis of various techniques and by the contribution of different scientists such as M. W. Nirenberg, P. Lader, H. G. Khorana, S. Ochoa, H. Ghosh, I. Gupta, T. M. Jacob, R. Morgan, R. D. Wells, P. Lengyel, J. F. Speyer etc., a complete genetic code dictionary could be prepared as given in the next page.

The m RNA genetic code dictionary

Codons	*Coded for Amino acid*	*Codons*	*Coded for amino acid*
UUU	Phenylalanine	UAU	Tyrosine
UUC	,,	UAC	,,
UUA	Leucine	UAA	Ochre (*Terminator*)
UUG	,,	UAG	Amber (*Terminator*)
CUU	,,	CAU	Histidine
CUC	,,	CAC	,,
CUA	,,	CAA	Glutamine
CUG	,,	CAG	,,
AUU	Isoleucine	AAU	Aspargine
AUC	,,	AAC	,,
AUA	,,	AAA	Lysine
AUG	Methionine (*initiater*)	AAG	,,
GUU	Valine	GAU	Aspartic acid
GUC	,,	GAC	,,
GUA	,,	GAA	Glutamic acid
GUG	,,	GAG	,,
UCU	Serine	UGU	Cysteine
UCC	,,	UGC	,,
UCA	,,	UGA	*Terminator*
UCG	,,	UGG	Tryptophan
CCU	Proline	CGU	Arginine
CCC	,,	CGC	,,
CCA	,,	CGA	,,
CCG	,,	CGC	,,
ACU	Threonine	AGU	Serine
ACC	,,	AGC	,,
ACA	,,	AGA	Argenine
ACG	,,	AGG	,,
GCU	Alanine	GGU	Glycine
GCC	,,	GGC	,,
GCA	,,	GGA	,,
GCC	,,	GGG	,,

Nature of Genetic Code

1. THE CODE IS A TRIPLET : A triplet or codon consists of three nucleotides of *m RNA* which codes for one amino acid. Therefore, there are 64 triplets or codons as mentioned in the genetic codon's dictionary.

2. SOME CODES ARE PARTICULATE : Some codons show one to one relationship between amino acid and the codon, e.g., methionine and tryptophan are coded only by *AUG* and *UGG* codons, respectively.

3. THE CODE IS DEGENERATE : A perusal of dictionary reveals that in certain cases for a particular amino acid, there are more than one codon, e.g., the amino acid, serine may be coded by either one of the codons *UCU*, *UCC*, *UCA*, and *UCG*. This accommodates the utilisation of additional codons of the 64 types.

4. SOME CODES ARE NONSENSE : Some codons such as *UAA*, *UGA* and *UAG* are nonsense as they do not code for any amino acid. But nothing in nature is useless and therefore it is improper to call them so. However, the importance of these codons have been quite recently established that these are in fact chain termination codons. The codon *UAA* is popularly referred as ochre and *UAG* as amber. When these codons are present in *m RNA*, these bring about termination of the polypeptide chain which would be then released for executing its function.

5. THE CODE IS NON-OVERLAPPING : It is to indicate that no nucleotide (or no letter of any codon) is used for two codons.

6. THE CODE IS COMMALESS : The codons are continuous and no punctuations are present in between the two codons. That is to say that second begins immediately after the first codon. The process runs automatically and no letters are wasted between the two codons to tell that one amino acid has been coded.

7. THE CODE IS NON-AMBIGUOUS : The coding property attributed to every codon is definite and as such there is no ambiguity about any codon. Though the same amino acid may by coded by more than one codon yet the same codon shall never code for two different amino acids.

8. THE CODE IS UNIVERSAL : While the very existence of *m RNA* codons dictionary has been mainly as an outcome from the work on micro-organisms (*Escherichia coli*), there is no doubt that in all kinds of living organisms the same genetic code is used. It obviously displays the inherent masterly skilled secret of nature.

9. CHAIN INITIATION AND CHAIN TERMINATION CODONS ARE DEFINITE : (*A*) *Initiation* : In almost every case of the experiments so far performed, it has been found that initiating amino acid is always methionine. The codon for which is generally *AUG* but rarely *GUG* has also been recorded. Surprisingly when *GUG* acts as initiating codon, it does not code for valine (as it should be according to the genetic code dictionary) but instead codes for methionine. However, Marcker (1965) states that the initiating methionine amino acid molecule is always formulated (as described under Protein Synthesis)

which distinguishes it from non-formylated methionine present elsewhere in the polypeptide chain. The *t RNAs* of both methionine are separate. However, this dual behaviour of codon *GUG* is a challenge to the concept that the code is unambiguous.

(*B*) *Termination* : The polypeptide chain termination is being brought about by either one of the codons—*UAA* (ochre) or *UAG* (amber). The terms ochre and amber refer to mutational studies made for the purpose. Benner and Stretton (1965) observed that amber and ochre codons cause termination of polypeptide chain not by producing a shortened messenger *RNA* in transcription from *DNA* but interestingly during translation from *m RNA* to polypeptide. Later Khorana (1966) added *UGA* in the list of terminating codons. It was also confirmed by Brenner (1967) that *UGA* did not stand for any amino acid. However, in view of the lack of strong suppressors for *UAA* (in contrast to the other two triplets), it has been suggested that *UAA* was the triplet commonly used by *Escherichia coli* to signal the end of polypeptide.

10. GENERAL : Visualising above facts, sixty one triplets out of 64 are allocated to amino acid coding and the remaining three act as stop signals in polypeptide synthesis. Studies made to establish the mechanism for constructing a polypeptide from the information encoded in nucleic acid reveals that several hundred different protein molecules are needed in order to synthesise even one such protein. Furthermore, these proteins, besides participating in the various ribosomal activities, remain involved in the formation of peptide bond itself, catalysing transcription, methylation, synthesis of amino acids, attachment of each amino acid to its transfer *RNA* etc. which in all evolve the brain scratching riddles to the enthusiastic scientists of molecular world.

GENERAL QUESTIONS

1. Write an essay on genetic code.

2. What is codons' dictionary and how different nucleotides direct the amino acids to protein synthesis ?

■ ■ ■

CHAPTER 23

REGULATION OF GENE ACTIVITY

In each cell of an organism, thousands of genes are present and not all of these are active simultaneously, that is to say that a gene is not active throughout the life time of the organism. There is a particular time for every gene to become active and to produce required enzyme. For example the enzymes which are required at seedling or embryo stage of an organism may not be required at the time of flowering or maturity. The unequal distribution of different structures such as hairs in a definite region of the body, nails in the other regions, leafs and flowers in the separate regions of the plant etc. are due to regulated activity of genes. Had there been all the genes active simultaneously, all the cells of a plant should have chlorophyll, root hairs, stomata etc.

Since all cells in a higher organism possess the same genome, such examples serve as prenote to consider the presence of some regulatory systems controlling the genic activites. Scientists kept on searching the solution to this most sophisticated and amazing gift of nature present in each cell. Studies made so far reveal that there are two sites of regulation of genic activities and controlling mechanism must be present separately. The one mechanism is supposed to be present somewhere in the cytoplasm and the other in the nucleus. The mechanism present in the cytoplasm would be regulating the synthesis or activity of an enzyme and that present in the nucleus must be regulating the activity of structural genes.

1. Regulatory Activities in the Cytoplasm

Under this category, either the activity of *m RNA* or the activity of an enzyme is regulated by some cytoplasmic metabolite or by the addition of some specific substrate. Examples like that of tryptophan synthetase, methionine synthetase or isoleucine synthetase are well known belonging to this category. If tryptophan or methionine is supplied to the culture of *Escherichia coli*, the activity of tryptophan synthetase or methionine synthetase slows down. The phenomenon may be referred as **enzyme repression** which is explained on the basis that the accumulation of end product of metabolic pathway inhibits the synthesis of enzyme involved in the process. Reverse to this case, the examples of phenomenon of enzyme induction have also been known in certain micro-organisms in which case the addition of certain specific substances can increase the rate of a synthesis of enzyme.

The example of inhibition of synthesis of isoleucine synthetase is more clearly known and the phenomenon is called the **feed back inhibition**. In this case, the end product accumulates and thereby stops further synthesis of this substance. It has been seen in *E. coli* where if normally growing cells are supplied with amino acid isoleucine, it blocks the activity of the enzyme involved in the first step. It in fact occurs due to binding of isoleucine to the enzyme threonine deaminase as follows.

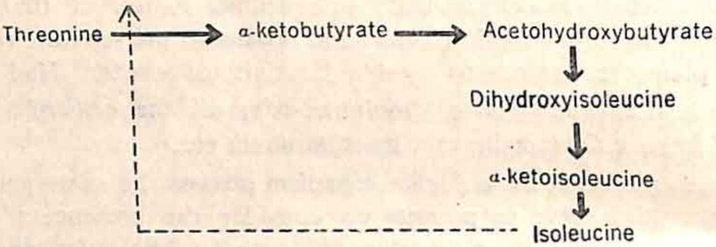


Fig 23.1. Feed back inhibition in the isoleucine synthesis.

The example is little different from enzyme repressor mechanism in the sense that in the feed back inhibition, the enzyme does not function while in the enzyme repression the synthesis of enzyme is stopped. An attempt to explain feed back inhibition mechanism involves that end product, perhaps, acts through allosteric transformation of the enzyme which makes enzyme incapable to perform its catalytic function.

2. Regulatory Activities in the Nucleus

Studies made on inducible system for the synthesis of β-galactosidase enzyme in *E. coli* by Nobel laureates Jacob and Monad (1961) led them to conclude that all genes do not code for the synthesis of enzyme proteins. Some of them are involved to perform other functions. They postulated that there is a separate class of gene whose sole function is to control and regulate the activity of a structural gene that codes for the synthesis of great variety of cellular enzymes. Genes of such a class are called **regulator genes** and they determine whether a given structural gene or a linked group of such structural genes will or will not form *m RNA* at a given time.

However, they proposed a model, called **operon model.** They observed that in *E. coli*, in the absence of lactose, β-galactosidase is not synthesised. Here lactose acts as **inducer,** in the absence of which genes responsible for the synthesis of β-galactosidase enzyme do not function. To explain this step, the presence of **repressor** capable to check the activities of the genes was proposed and now have been verified. The activities of such repressor are controlled by the synthesis of inducer, i.e., it can be rendered inactive by the addition of corepressor. Thus the model visualises the presence of two systems.

(i) *Inducible system* : which involves the inactivation of repressor by the presence of inducer (i.e. Active repressor + Inducer→ Inactive repressor). When repressors are inactive structural genes become functional and begin to synthesise *m RNAs* and thereby enzymes to regulate metabolic activities.

(ii) *Repressible system* : which involves activation of repressor due to presence of corepressor (i.e. Inactive repressor + corepressor→ Active repressor). When repressors are active, the activities of structural genes remain ceased and there is no synthesis of *m RNA*.

The following map of **Lactose repressible system** will elucidate the set up of operon model more properly.

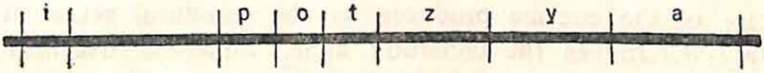


Fig. 23.2. Lactose operon model of *Escherichia* coli [*i*=regulator gene (for repressor), *p*=promoter gene (for transcription initiation), *o*=operator gene (for the action of repressor), *t*=translation initiation site, *z*=structural gene for β-galactosidase, *y*=structural gene for β-galactosidase permease and *a*=structural gene for acetylase.] Operon consists of *p*, *o*, *t*, *z*, *y* and *a* genes and controlling genes are *i*, *p*, *o*, and *t* (Redrawn from Watson, 1965).

In the model, repressor is synthesised through the activity of a gene, *i* (regulator gene). The repressor then attaches on operator gene, *o*, to check the activities of structural genes, *z*, *y* and *a* and there is no synthesis of *m RNAs* (fig. 23.3).

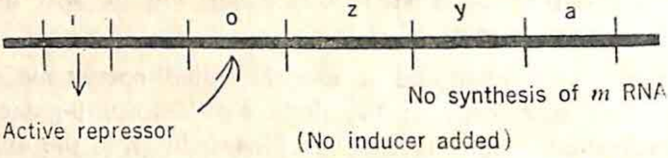


Fig. 23.3. Operon showing the action of repressor (see text).

By the addition of lactose (inducer), repressor is made inactive which fails to attach on the operator gene. As a result, structural genes become active and the synthesis of *m RNA* begins.

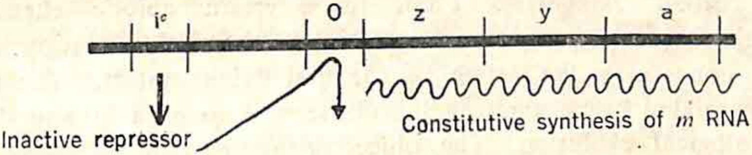
In the model, the operator gene is the site of action of repressor, the promoter gene is the actual site of initiation of transcription process and structural genes are the site of actual transcription process.

More detailed studies with sophisticated modern techniques to establish the nature of various genes of the operon model has been made. The inducer is often natural substrate of the enzyme which the structural gene causes to be produced. In the absence of substrate, the production of its enzyme is purposeless. So the presence of repression mechanism is safeguard against wastage. The repressor molecule is usually a protein which may be excercising toxic effect to the cell. In presence of abundance of repressor molecules, the regulator gene represses the functioning of structural genes. A regulator gene is thus different from structural genes and these may be present in a separate chromosome. It does not change the nature of the enzyme produced by the structural genes rather it simply determines the condition under which the structural genes will produce enzymes.

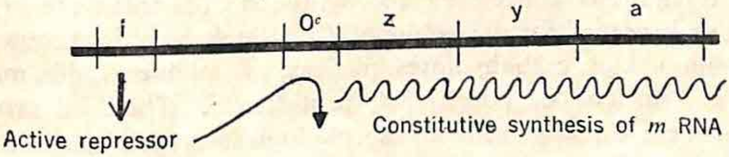
Besides, the presence of such a regulatory operon model in higher plants with eukaryotic cells where large number of chromosomes are present remains still to be explored authentically. It has been visualised that some cytoplasmic enzymes such as histonases play a major role in removing the non-histone proteins of *DNA* and thereby to activate the repressed *DNA* in the synthesis of *m RNA*. Britton and Davidson (1961) have suggested that the hormones and vitamins may be involved in the simultaneous activation of various genes. These substances may be referred as activators. Besides, the activator *RNAs* may also be present within the nucleus. It has also been visualised that in a *DNA* molecule there may be repeated segments acting as **receptors** for various inducers or activators.

3. Mutational studies

Mutational studies on the regulatory gene "*i*" and operator gene of the operon model has been made. It has been found that a mutation may result in the absence of repressor which may result into an unchecked synthesis of enzyme. Such strains associated with this property are referred as **constitutive strains** or **constitutive mutants.** A mutation does not allow the mutated gene to act, e. g., a mutation in the operator gene does not allow repressor to execute its function of controlling the operator site (fig. 23.4 B). However, based on these studies two types of constitutive strains are recognised : (a) regulator constitutive and (b) operator constitutive.



(A) Regulator constitutive



(B) Operator constitutive

Fig. 23.4. Diagrams elucidating concept of constitutive mutants. (A) Regulator constitutive (mutation in repressor gene makes it inactive) and (B) Operator constitutive (mutation in operator gene makes it unable to accept the command of repressor, i.e., active repressor fails to attach on operator site) (Redrawn from Watson, 1965).

GENERAL QUESTION

1. Discuss critically how there is a regulation over gene activities.

■ ■ ■

CHAPTER 24

ORIGIN OF LIFE

The subject origin of life has been of immense importance to mankind not only in view to understand the actual mechanism of the process but also to explore the mystery of nature. The nature is still an unsolved problem of great anxiety and a challenge to so called most intelligent human race.

The modern scientists have concentrated in resolving three major steps about origin of life, i.e., they have visualised the problems in three broad categories. First, to ascertain abiotic chemical evolution, second, leading to pre-biotic chemical evolution and third, concerning the origin of the first living system. The first is thus related to inorganic evolution, second to organic and third to biological evolution. The object of the first two problems is to prepare the background for the third. These include experiments and explanations concerning the synthesis of inorganic to organic compounds needed for the origin of life. Such organic compounds are—amino acids, carbohydrates, purines, pyrimidines, lipids, nucleosides and nucleotides, porphyrins, peptides etc. The third problem visualises the establishment of morphological models and then to genetic model of precellular life. Once the origin of primordial germ is established, the further evolution of complicated modern life forms can be explained on the basis of Darwinian concept (organic evolution).

The whole of the approach is based on evolutionary philosophy and the phenomenon of interest may be considered as a development forward from the primeval cloud of hydrogen gas to the living organism of today. The earth is about 4·5 billion years old and therefore the problem is to find the history of this planet from the time of its birth until the emergence of life. That is to say that scientists wish to know some thing of our remotest ancestry.

Considering 10^{20} stars in whole of the universe, Shapley (1958) estimated that there must be atleast 10^{8} planetary system suitable for life. Thus, life can be considered to be an inevitable consequence of evolution of matter. Morgulis (1953) wrote "The origin of life was not an occurrence ascribed to some definite place and time. It was a gradual process of unfolding which consumed perhaps more million years than were required for the evolution of all the species of living things. A long chemical evolution was considered necessary for the origin of life." The chemical evolution, probably, began with the very origin of matter and highly reactive elements, which occur in living organisms, probably existed in their reduced form, e.g. carbon as methane, nitrogen as ammonia and oxygen in water.

When the earth aggregated about 4·5 billion years ago from the primitive dust cloud, these rudimentary molecules were perhaps already in existence. Within this framework, life appears to be a special property of matter which arose at a particular period in the existence of our planet. Close to this opinion, Oparin (1924) suggested that there was no fundamental difference between a living organism and brute matter. The complex combination of manifestations and properties so characteristic of life must have arisen gradually in the process of evolution of matter. However, some of the important steps suggesting various schemes for the origin of organisation of pre-biological systems are outlined here.

1. Chemical Evolution

Studies based on astronomical spectroscopy reveals that with surprising uniformity the most abundant elements in the universe are in the orders of ranks—hydrogen, helium, oxygen, nitrogen and carbon. Of these, H, N, O and C are the basic constituents of living system. However, rarity of noble gases (helium) from earth atmosphere and their cosmic distribution suggest that primary atmosphere of earth was almost completely lost in early times. The present atmosphere is of secondary origin, the chemical composition of which must have been similar to that of primary atmosphere. Study of chemical equilibrium shows that in excess of hydrogen, the elements C, N and O were present to their reduced form as methane, ammonia and water. One of the examples for the formation of ammonia may be due to the reaction between metal nitrides and superheated steam.

$$MeN + 3H_2O \rightarrow Me(OH)_3 + NH_3 \ (Me = \text{metal})$$

Energy sources for the synthesis of organic compounds under primitive earth conditions were UV from sun, electrical discharges, ionising radiations and heat.

2. Pre-Biotic Chemical Evolution

The probable origin of few important different organic compounds needed for the origin of life are discussed here.

(A) AMINO ACIDS : Of the many experiments made and explanations proposed, Miller's experiment is by far the most important and convincing. Stanely L. Miller (1953) exposed a mixture of methane, ammonia, water and hydrogen to an electric discharge for approximately one week. As a result many organic compounds were formed and among those that could be identified were glycine, alanine, β-alanine, aspartic acid and glutamic acid (amino acids). He offered two proposals explaining the mechanism of formation of amino acids— (i) the aldehyde and hydrogen cyanide which could have been synthesised in the gas phase would react further in aqueous phase to produce amino and hydrogen acids, (ii) these acids were synthesised directly from ions and radicals produced in the gas phase. It is believed that both these processes might have played role in the accumulation of amino acids.

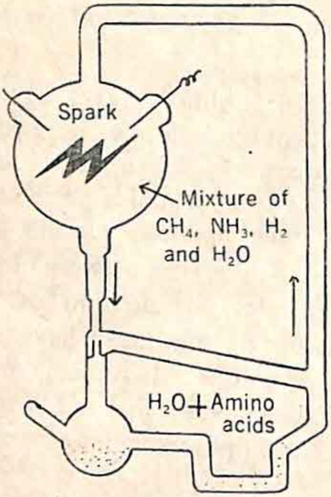


Fig. 24.1. Miller's experiment on origin of life.

After Miller's experiment, hundreds of other experiments were carried out. Ponnamperuma and Flores (1966) were able to obtain conversion of more than 95% of methane into organic compounds. Harada and Fox (1964) conducted a typical experiment. They passed a mixture of methane, ammonia and water over quartz sand or alumina in a glass reaction tube held at a temperature 900-1000°C and found fourteen different amino acids as end products. This experiment was to formulate that volcanism and possible residual heat in the earth's crust might have also been responsible for the appearance of amino acids.

(B) CARBOHYDRATES : From the hypothetical primordial gas mixture, neither monosaccharides nor polysaccharides have been detected as products of reaction. Palm and Calvin (1962) and Ponnamperuma and Flores (1966) reported that formeldehyde is formed when methane, ammonia and water mixture is subjected to an electric discharge or ionising radiation. It has been further shown that *UV* irradiation of formeldehyde can lead to formation of detectable amounts of ribose and deoxyribose as well as other sugars such as 2-deoxyribose. Likewise, aqueous solution of various concentrations of formeldehyde were refluxed with suspended kaolinite. The products were separated into trioses, tetroses, pentoses and hexoses.

(C) PURINES AND PYRIMIDINES : Oro (1960) for the first time demonstrated the synthesis of adenine from ammonium cyanide. The latter was abundant on the surface of primitive earth. However, this fact was established by the work of Ponnamperuma et al (1963), when they could form adenine by electron irradiation of methane, ammonia and water. It was further shown that *UV* irradiation of dilute solutions of hydrogen cyanide also produced adenine and guanine.

(D) NUCLEOSIDES AND NUCLEOTIDES : Sagan (1961) and others speculated that photoactivation of the purines could have played a role in the synthesis of nucleotides. When a dilute solution of adenine, ribose and sodium orthophosphate was exposed to *UV* light, a detectable quantity of nucleosides was formed. On further irradiation at wave length 2400 A° and 2900 A°, the synthesis of nucleotides was also detected. These experiments acccount for the possible synthesis of nucleotides in the primordial environment.

(E) PORPHYRINS : Oparin's hypothesis of origin of life reveals that first organisms were heterotrophs which utilised the organic material. These became depleted and the environment gradually shifted to an oxidising environment, the autotrophism developed. For accepting this fact, porphyrins and their precursors must have been present in the abiogenically produced primordial organic substances. Hodgson and other (1968) have shown synthesis of pre-biotic porphyrin from methane, ammonia and water applying electric discharge method.

(F) PROTEINS : Once the problem of synthesis of amino acids from primordial environment is established, the problem remains about the polymerisation of amino acids for which many hypotheses have been put forward. Fox (1960) believes that polymerisation of these amino acids is generally affected at 180-200°C. Kliss and Mathews (1963) and Mathews and Moser (1966) proposed that hydrogen cyanide dimerises to aminocynomethylene $H_2N-C=C=N$, which would have led to polymeric peptide precursors. They further substantiated this fact by other experiments. It was found that when methane and ammonia were subjected to electric discharge and the residue heated with dilute acid, the presence of polypeptide was found.

The formation of polypeptide occurred through natural polymerisation which led to develop into the origin of macromolecules. It is presumed that these macromolecules aggregated on water surface to give rise to the monomolecular layer and then became folded. After attaining a certain weight, the sinking of these macromolecules occurred from which further complex biological macromolecules were formed.

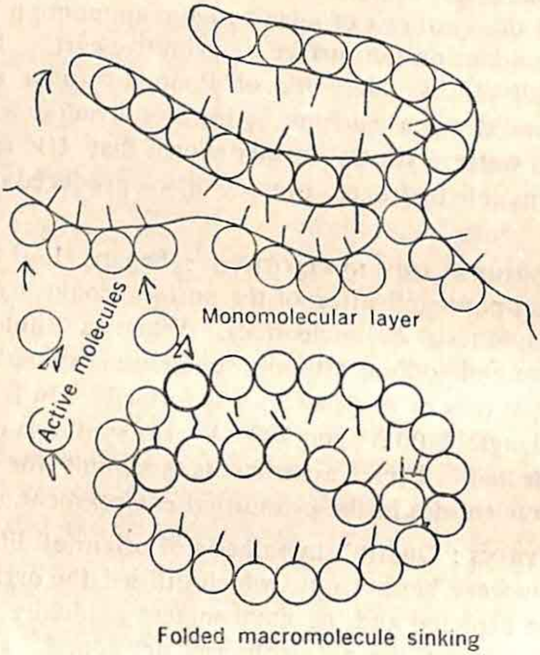


Fig. 24.2. Hypothesis to visualise the possible method of origin of biological macromolecules.

3. Origin of First Living System

The above discussion reveals the abiogenic formation of abundant organic compounds. Therefore, problem remains to speculate that how this organic matter was organised into free living and self reproducing units which may follow Darwinian or any other theory of evolution to account for the different cellular types known today. In fact this aspect comprises of greatest gap in present knowledge which can reveal the origin of first living system in true sense.

Visualising the first morphological models of precellular life, Oparin (1964) and Fox (1965) proposed two different theories. Both agreed to units of life as multimolecular aggregates of macromolecules. Oparin promulgated coacervate drops while Fox synthesised protein microspheres.

COACERVATE DROPS : Coacervation is a phase separation that can occur in a variety of colloidal sols. Oparin suggested that by coacervation abiogenic organic matter concentrated in the primitive ocean. Such coacervates retained their structural integrity and thereby provided minute environments in which chemical reactions

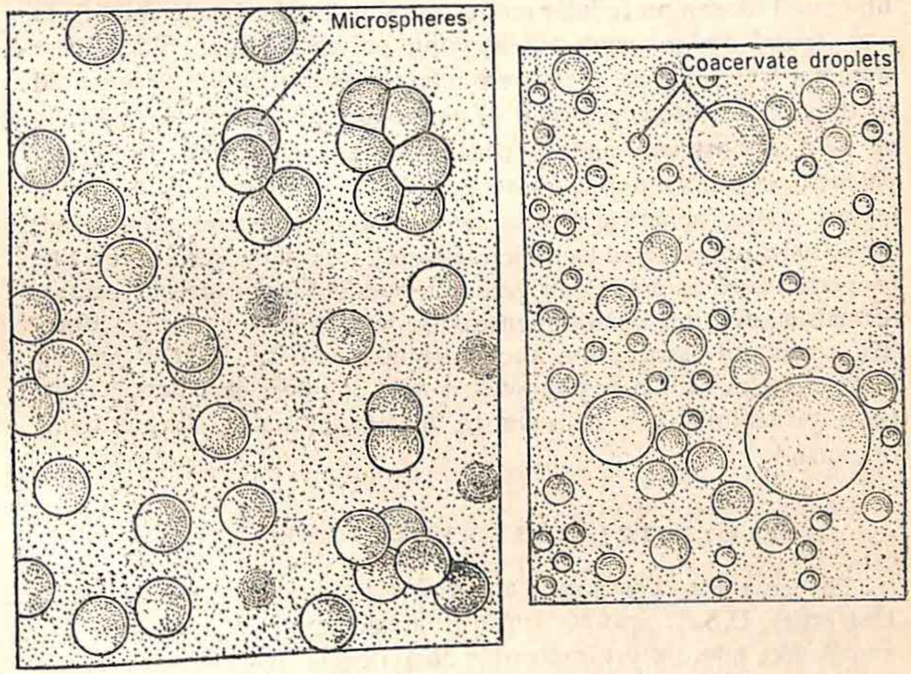


Fig. 24.3. Figure showing microspheres and coacervate droplets. For detail see text.

proceeded more rapidly. He verified this hypothesis in principle by constructing enzyme-coacervate drops with polymers of biological origin and these became evolutionarly complex material. The coacervate must have been subjected to variable increase in their size, either through the synthesis of new material within them or by the incorporation of additional soluble organic matter. Oparin assumed that coacervate drops of different compositions would have increased in number as well as in structure so that a selection of 'most fit' would have ensued.

This hypothesis faced two serious objections. Firstly, the coacervate drops have been found too unstable to be useful cellular model. Secondly, these require macromolecules for their formation.

PROTEINOID MICROSPHERES : Fox's proteinoid microspheres is the other well known precellular model. He simulated volcanic conditions and found amino acids undergoing polymerisation. Since these were not true proteins, these were referred as proteinoid. Proteinoid is thus an anhydrous polymer of amino acids produced thermally in an inert atmosphere and is considered as a possible primordial precursor of contemporary proteins. When proteinoid is dissolved in hot water, spherical bodies less than 2μ in diameter are formed. These units are referred as "microspheres" and show certain cell-like properties such as growth in size of bud-like appendages, septale formation, division, osmotic sensitivity, bilamellar boundaries, algae-like structural associations and hormonal activity. These can also make complex with deoxyribonucleic acid or ribonucleic acid. Thus, these proteinoids are believed to have given rise to the first living organisms.

ARTIFICIAL SYNTHESIS OF LIFE

The news about successful artificial synthesis of life from Stanford University, U.S.A., was acclaimed from all corners of the world. It sounds like man's most impossible dream come true. Nobel laureate Arthur Kornberg and biochemist Mehran Goulian synthesised a virus that can infect bacteria and reproduce itself. They selected four nucleotides and added a *DNA* polymerase enzyme to it. The enzyme helps nucleotides to assemble into typical *DNA* helix-shaped strand. They selected the *DNA* templete of *Phi X* (virus) to guide the assembly of synthetic molecule. As the polymerase acted, the four nucleotides aligned themselves in code-like combination along the natural *DNA*, eventually forming a strand of 6,000 nucleotide units. The replica became the templete to produce a man-made duplicate of the natural *DNA*. This *DNA* synthesis is obviously one more bold step ahead towards the complete understanding. Later, H.G. Khorana (1972) also succeeded in synthesising the gene capable to produce specific transfer *RNA* (*t RNA*). All these modern achievements reveal that the riddle concerned with origin of life may soon be successfully solved.

GENERAL QUESTIONS

1. Discuss critically the mechanism of origin of life.

2. How would you account that nature advanced to living objects from non-living matter ? Explain.

2. How far do you agree about scientific explanations related to origin of life.

4. Write short notes on—

(i) Miller's experiment, (ii) Origin of macromolecules,
(iii) Natural origin of nucleic acid and proteins.

■ ■ ■

CHAPTER 25

EVIDENCES OF EVOLUTION

From the very inception of the fact that life arose by a series of progressive chemical reactions, the concept of evolution was given a great importance. It is now universally accepted that evolution has occurred among plants and animals. In other words, the present day plants and animals have descended from simpler forms those existed on the earth in former times and will in course of time produce new forms. Evolution is a very very slow process which cannot be observed as we can observe the things and events around us. The concept of evolution explains that—(i) the present day forms have descended from different types of living beings of the past, (ii) the evolution has been very slow, gradual and continuous but at present several links have been broken because of extinction of various forms, (iii) there have been divergent changes which with the passage of time has given more and more diversities and (iv) the causes of inducing diversity are even present today and active.

The fact that evolution occurred has been substantiated by several evidences. A few of them are enlisted here.

1. Evidences from Embryology

All sexually reproducing forms begin their life from one celled zygote which, in general, by a process of cell division enters into the embryonic stage. The study of the comparative embryology provides convincing evidences in support of evolution. Such evidences came into prominence after Haeckel who proposed the **biogenetic law**, also called **recapitulation theory**. The theory envisages that 'ontogeny recapitulates phylogeny' which means that embryos in their development repeat the evolutionary history of their ancestors in an abbreviated form. Thus, Haeckel believed that embryonic stages correspond to ancestral adults which provide evidence for the possible line of organic evolution. For example, the mosses with their filamentous protonema indicates (according to biogenetic law) their algal ancestry. Likewise, many animals show characters of early stages of development that resemble the characters of ancestors.

In order to elucidate the close resemblances, the various stages of embryology of different vertebrates are given in fig. 25.1.

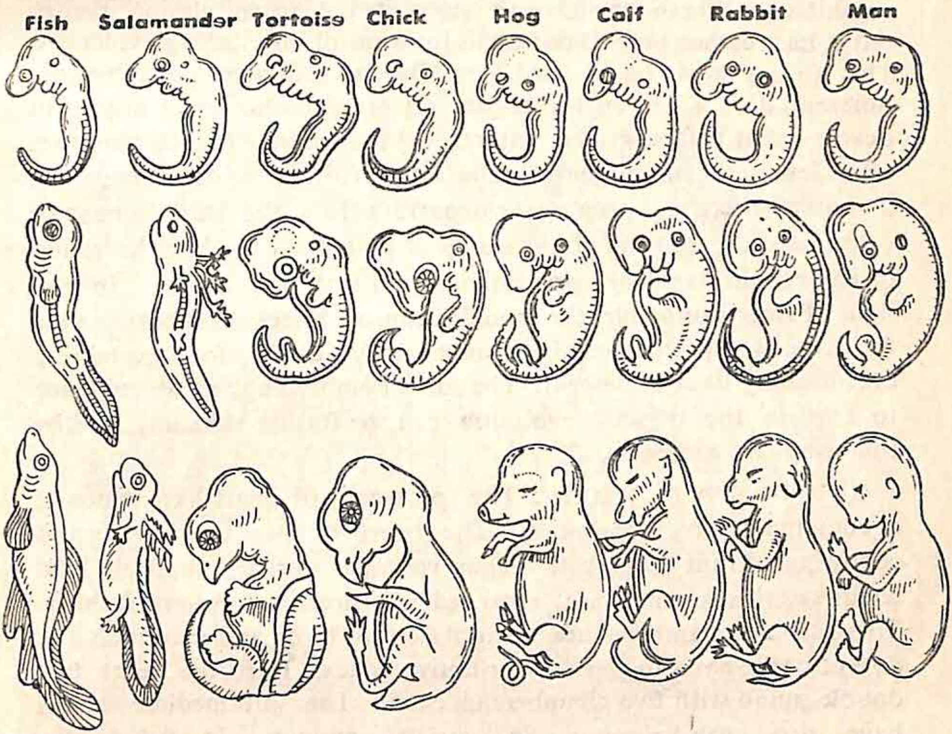


Fig. 25.1. Figure showing early stages of development of embryo of various vertebrates.

The tadpole of a frog is very much like a fish. It swims and breathes similarly. It indicates that frogs have been evolved from fish-like ancestors. Similarly the presence of gill slits in mammalian embryos indicates fish-like ancestry for the mammals. A progressive sequence of evolution of kidney can also be traced on the basis of different examples. The primitive vertebrates like hag-fishes have pronephric kidneys, the amphibians and adult fishes have mesonephric kidneys while in birds and mammals during early stages the earlier described both types of kidneys are formed which are soon replaced by metanephric functional kidneys of adult organisms. The reason for the presence of two types of kidneys and then replaced by third type provides an assumption that higher vertebrates have evolved from lower ones.

The perusal of figure 25.1 reveals that there are so much similarities between the embryos of different forms that in early stages it is not easy to distinguish a human embryo from that of an amphibian. These facts though support the recapitulation theory but it has fetched several criticisms for want of interlinking evidences. This theory was based on Von Baer's principles of embryonic differentiation which envisages that (i) general characters appear in development before special features, (ii) the special character appears after less and more general characters, (iii) during development in an animal there is a progressive departure from the development of other animals, and (iv) young stages of an animal are like the young or embryonic stages of lower animals and not like adults. In the light of Baer's principle, the modification of Haeckel's theory makes the results of comparative embryology useful for explaining evolutionary lines of descent. The role of embryology as an evidence to explain the organic evolution can be further substantiated by following two examples.

(i) ONTOGENY OF HEARTS : The presence of heart-like structure is common among vertebrates. The heart of primitive vertebrates comprises of four chambers—sinus venosus, auricle, ventricle and conus arteriosus which are arranged in posterioanterior fashion. These four chambers are also present in birds and mammals but do not correspond to those of primitive ones. Here the heart is a double pump with two chambers in each. The intermediate stages have also been observed in various animals. In fishes, the differentiation of heart stops before any subdivision of auricle or ventricle appears but in mammals further differentiation leads to the subdivision of auricle and ventricle. There remains original single opening between them. Besides, during ontogeny absorption of sinus venosus into the right auricle and a division of conus arteriosus into pulmonary trunk leading from the right ventricle and a major aorta from the left ventricle also take place. These changes probably appeared in response of breathing adaptations.

(ii) ONTOGENY OF AORTIC ARCHES : Like hearts, the ontogeny of aortic arches also support the concept of evolution. In embryo stage, all vertebrates including mammals have six aortic arches running from ventral aorta to dorsal aorta. But in mammals, during development, these arches are dropped out so much so that only three remain in mature mammals. The persisting ones are the *third arch* supplying to head region, the *fourth arch* supplying to systematic circulation and the *sixth arch* supplying to lungs. In fishes, however, six arches are modified in various ways but those of *Choanichthyes* (a fish) are closely related to amphibians, i.e., the first arch is dropped out in adults. The evolution of aortic arches in different classes are shown in fig. 25.2 which explains that during ontogeny there is a recapitulation of embryonic stages of primitive forms.

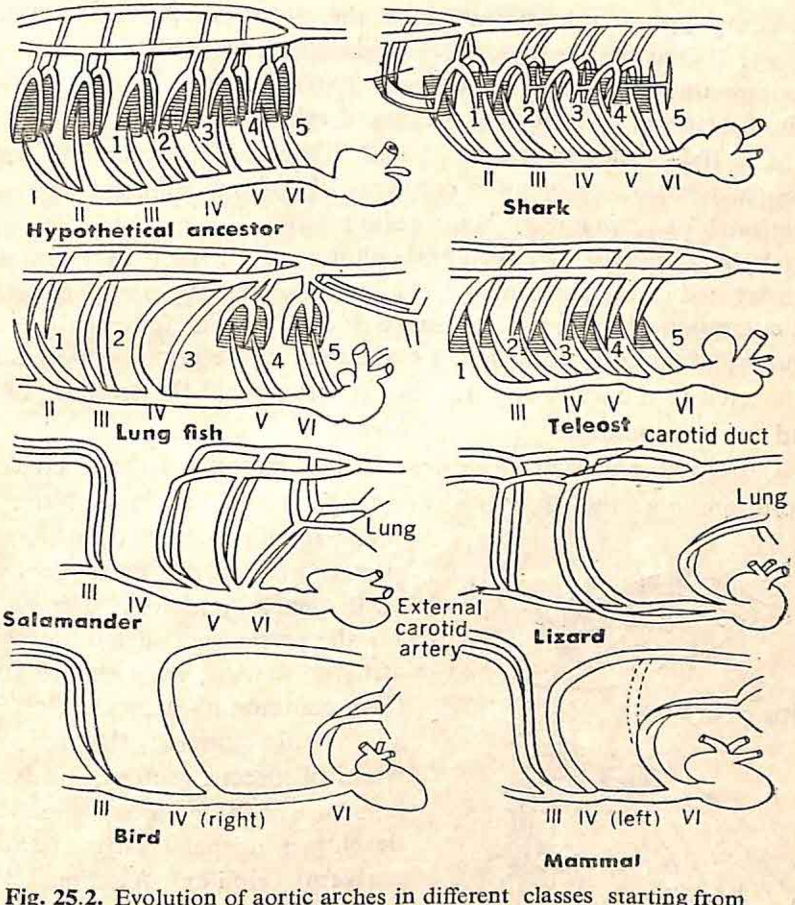


Fig. 25.2. Evolution of aortic arches in different classes starting from hypothetical ancestor (Redrawn from Dodson, 1960).

These two examples fairly support the recapitulation theory and likewise several other examples in animals are observed. However, examples to support such a theory are rare in plants, probably because of simpler embryology. In *Acacia*, the leaves are simple at seedling stage while compound in tree stage. In cacti, leaves are absent in adults but commonly present in young stage. Yet another good example is that of conifers. If a wound is made in a coniferous wood, the new growth differs histologically which can be compared to the fossil coniferous wood of the mesozoic era.

2. Evidences from Comparative Anatomy

Comparative anatomy extends the strong support to evolution theory. One can very safely feel that cells and tissues are the fundamental and similar structures that make up the body of an animal or a plant. Also that a marked relationship between different ferns in their stelar organisation and likewise the flower in angiosperms reveals the similar concept. In them, different degree of relationships can be recognised quite easily.

Among animals, the vertebral column in different vertebrates is constructed in the same plan. The muscles and the digestive systems all correspond in them. The large blood vessels follow the same course giving off branches at essentially corresponding points and connected to the same organs. So is true with the presence of eye and ear in mammals.

Certain homologous structures further strengthen the doctrine of evolution, e.g. the fore limbs of frog, lizards, bat, birds, horse and man are all constructed on the same pattern and include the same bones in the same position. The reason for the presence of such homologous structures is that they are descended from common ancestors. Similarly, analogy also supports the view. The wings of insects, reptiles, birds and bats are analogous as these have developed independently. Since these perform similar function, these appear identical morphologically. But the wings of an insect is a membrane supported by veins, those of birds and bats are derived from fore limbs and are bony. The resemblance is due to analogy which is only superficial. It is believed that it is the result of adaptation to more or less similar conditions of the environment. They do not indicate

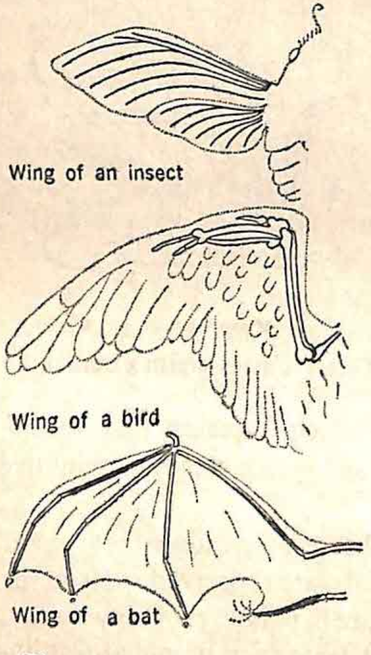


Fig. 25.3. Figure explaining analogy between wings of insect, bird and a bat.

relationships. Analogy thus reveals that envionment has played a vital role in evolution.

3. Evidences from Vestigeal Organs

Quite many reduced and functionless organs are found in animals and plants. Such organs have been referred as vestigeal organs. Their presence in the living organisms is often considered as rudimentary examples of once well developed organs found in the ancestors. These have become atrophied due to alternate development in the organisms and change of environment.

Some well known examples of vestigeal organs are the presence of staminodes among members of families caesalpinioidae, cucurbitaceae, Labiatae, Scrophulariaceae and the leaves of *Cuscuta*, *Asparagus* and *Ruscus*. Among animals, the man alone has nearly 180 vestigeal structures. Some of them are quite commonly known such as the nictitating membrane, the coccyx at the lower end of vertebral

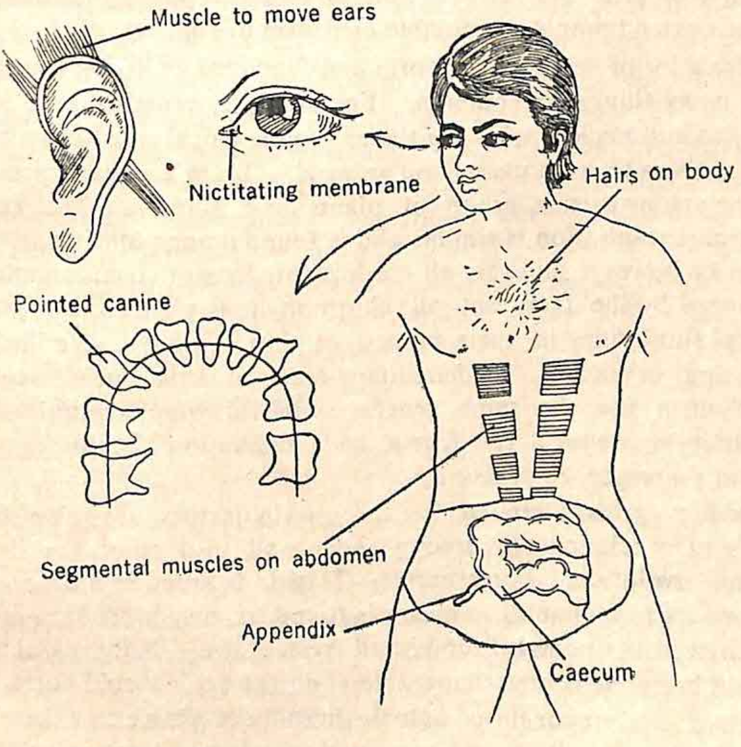


Fig. 25.4. The presence of vestigeal organ in man, some portions are shown separately.

column, vermiform appendix, hairs on body, segmental muscles on abdomen, pointed canine, muscle to move ears etc.

The nictitating membrane, a fold of skin in the inner corner of eye, is a vestige in a man whereas in several vertebrates it is well developed and is used for cleansing the cornea. Similarly, the vermiform appendix is a blind finger-shaped process in man which is vestigeal but in other herbivorous mammals it is well developed that helps in the digestion of cellulose.

Some other examples of vestigeal organs found in animals are —the splint bones of horses, the degenerate eyes of aquatic animals living in darkness, reduced wings in kiwis etc. The presence of such vestigeal organs cannot be ignored assuming that these are formed useless organs. There are reasonably sound evidences to account for descent of vestigeal organ possessing organisms from ancestors which have these structures functional.

4. Evidences from Physiology

Physiological similarities and differences among plants and animals extend ample of valuable evidences in support of evolution. Besides a lot of variations in form and functions of living organisms, quite many things are common. For example, proteins, fats, carbohydrates and nucleic acids are the common biochemical constituents of organisms to both plants and animals. There are many processes similar among certain group of plants and animals. The general pattern of respiration is similar and is found among all living beings. It can be however said that all green plants have a common ancestry is evinced by the fact that all chlorophyllous plants show physiological similarities in their process of photosynthesis. Similarly in all living organisms, the hereditary material is the nucleic acid and all of them use the same genetic code for amplification of the hereditary messages. The genetic code differs in different organisms only in the sequence of bases.

Besides general similarities, group similarities are also found that confer relationship among them and also trace the line of organic evolution. For example—Trypsin is found in animals from protozoans to mammals; amylase is found from sponges to mammals and thyroid hormone is found in all vertebrates. Not only this but thyroid hormone is interchangeable, i. e., the beef thyroid hormone is useful in the treatment of human thyroid deficiencies. Likewise in plants also, certain hormones such as indole acetic acid and gibberellic acid are common.

Keeping these physiological similarities in view, serological tests based on the reaction of blood serum were carried out recently. These tests have also shown relationships. Further it has also been seen that human blood proteins are mostly like those of anthropoid apes and other primates establishing relationship between them. It can however be concluded that examples of basic physiological similarities offer evidences in support of concept of organic evolution.

5. Evidences from Genetics

The science of genetics extends direct evidences of evolution. Besides the presence of common hereditary material that controls the expression of traits and all the living processes, the studies on mutation unravels the mysteries of nature that how variations have been produced and new forms have adapted during the long course of evolution. That is to say that by mutational studies it has been possible to evolve new types, some of which are better suited in the environment. The recent examples of mutational new types successful to present environment are the dwarf mutations of wheat and rice and also the melanic forms of British moths.

A permanent heritable variation may also arise by chromosomal aberrations, polyploidy etc. Utilising polyploidy many new species of plants such as *Raphanobrassica* and *Triticale* have been synthesised. Similarly in nature also that polyploidy has played a key role in evolution can be substantiated considering examples of wheat, cotton, sorghum etc. Above all the most important genetic phenomenon that has played an elemental role in evolution is recombination. In general, the recombination induces diversity in new offsprings and on this fact, the natural selection works.

6. Evidences from Domestication

An additional support to evolution is deduced from domesticated animals and cultivated plants. These have been domesticated and cultivated since time immemorial. There have been a lot of changes among them indicating that evolution has occurred. It is a matter of common observation that certain plants which have been under cultivation for centuries have no trace left of their wild ancestors. In several cases these have become too distinct and different to compare each other. Cotton constitutes a good example. Fine fabrics discovered in the remains of Mohenjodaro indicate that as far back as 4,000 years it has been cultivated. The present day species are the modifications of the original wild one, produced through selection and polyploidy. Cytological studies on American cotton indicates that its an allotetraploid.

Among animals, dogs and horses are common examples of domestication. It is considered that about 200 varieties of dogs and 2,000 varieties of horses have evolved during 10,000 and 4,000 years of domestication, respectively. Likewise pigeons, rabbits, chicks etc. have been under domestication for centuries and now there are great varieties of them.

7. Evidences from Classification

The present day flora and fauna is represented by nearly 2,50,000 species of plants and 10,00,000 species of animals. Inspite of several diversities, on the basis of similarities it is possible to group these species into smaller or larger groups. Taxonomists have used the phenotypic characters in evaluating the systematic position of different forms. The basic unit of classification is the species which is an assemblage of all individuals of one kind. Hurst defined species as group of individuals of common descent with certain constant specific characters in common. The similar species are grouped under a genus, genera into a family, families into an order, orders into a class, classes into a phylum and phyla into a kindgom. Thus in classification there are groups within groups where the members of each group resemble each other more closely than those of other groups. It is to say that individuals of same species are strikingly similar so much so that these may be regarded as descendants of same parent. Likewise, different species of the same genus because of some similarities may be regarded that these are related, by descent from common ancestors. It may be true for genera in a family, families within order or so on. The classification is therefore based on similarities and differences arranging the various living beings in a sequence which indicates that more complex forms have evolved from simpler forms or ancestors.

8. Evidences from Geographical Distribution

The geographical distribution of living beings also extends the support to organic evolution. Charles Darwin in his voyage observed the distribution of same or similar species on widely separated land masses (which were once evolved) show that they have evolved as one unit and later on geographical isolation resulted into divergent evolution. He also noted that species separated by physical barriers, such as high mountains and seas over which migration of species is not possible, are quite different even if the environment is similar. For example, the deserts of Africa are full of Euphorbias while those of America are rich in cacti. Similarly elephants are seen in India and Africa alone and not in other tropical regions of the globe. Penguins are found in the South Pole while Polar Bears to North Pole, though both Poles have similar climates.

On the basis of similarities and dissimilarities of flora and fauna, the world has been divided into six biogeographical regions.

(i) Palearctic region (Europe, U.S.S.R. and China)
(ii) Nearctic region (North America and Greenland)
(iii) Ethiopian region (Africa and South of Sahara desert)
(iv) Oriental region (Asia and South of Himalayas)
(v) Neotropical region (Central and South America)
(vi) Australian region (Australia and neighbouring islands).

Each region has its own characteristic endemic flora and fauna. A careful perusal of this kind of distribution reveals that these regions are separated from one another by various physical barriers. Because of which even after keeping similar climate, these regions are inhabited by different types of plants and animals. This happened so because in different regions evolution occurred in different courses.

9. Evidences from Fossils

The fossils provide the most concrete evidences in support of evolution. These are naturally preserved organisms in rocks of various ages. In general, the organisms are destroyed by predators, scavengers or by micro-organisms after their death but a few escape destruction and get preserved as fossils. The most suitable environment for fossilisation is met within the ocean. Sometimes organisms are buried under volcanic lava and are preserved as fossils. However, the presence of antiseptic substances in water, low oxygen content and high toxic substances etc. prevent decay and help in preservation of fossils. There are many kinds of fossil forms. **Compressions** are the common type of preservations which represent those fossils which preserve much of organic matter of the plant in their impressions. These are quite helpful in furnishing information regarding external morphology of plants of past. Compressions of liverworts, ferns and *Ginkgo* leaves etc. have been observed in Yorkshire Coast of England. **Incrustations** may be defined as an external mold of a plant, usually in some uncompressible material.

Petrifactions include fossils preserving external form, internal structure and sometimes substances of original plants. They are best but rarest fossils. Occasionally organisms leave behind their impressions on mud or sand, which harden into stone in due course. These are called **moulds. Casts** are still other types of fossils in which case the cavities of impressions are filled with some hard material. Both moulds and casts are useful in determining the form and structure of many extinct species. Now, with the use of modern techniques such as 'radio active clocks' it is now possible to determine the age of fossils. All living beings contain a certain percentage of radio active elements which tell the time with this technique.

The fossil records tell the time when the major groups of organisms appeared. Though fossil records are poorly represented yet they have provided a lot of information about the fact and course of evolution of a variety of major groups of living beings. This fact can be visualised in the table 2 S. I. Geological time scale.

There are many examples of importance extending support to concept of evolution. Only two are discussed here.

(a) *Archaeopteryx*, A MISSING CONNECTING LINK : The reconstructed fossil bird *Archaeopteryx* serves as an example of link between reptiles and birds. This fossil bird of Mesozoic Era had a long tail and jaws full of teeth-like reptiles and wings with feathers like birds. Because of the presence of wings in preservations, these were referred as birds. Such a fossil provides a connecting link between the reptiles and birds suggesting that birds were evolved from animals like reptiles.

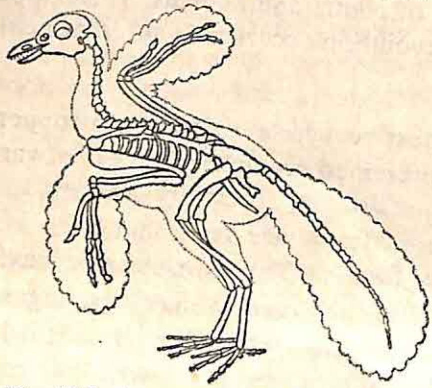


Fig. 25.5. A reconstructed fossil bird *Archaeopteryx*.

(b) EVOLUTION OF HORSE : While tracing the history of evolution of horses, several interesting aspects come across. The history dates back about 60 million years in the eocene period involving about 20 genera. It began with the advent of *Eohippus* in North America. *Eohippus* was of the size of a fox with four toes in front foot and only

[illegible] AND OTHERS

[illegible] etc.	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
Period [illegible] Glaciation	[illegible] mammals.	[illegible]
[illegible] climate with [illegible]	[illegible] of man	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible] mammals and birds [illegible] recent.	[illegible]
[illegible] than [illegible]	[illegible]	[illegible]
[illegible]	[illegible] of [illegible] mammals [illegible] plants [illegible] Arctic	[illegible]
[illegible]	Extinction of giant [illegible]	[illegible] present

three toes in hind foot. Each toe ended in a separate hoof-like nail. Their molar teeth were low crowned indicating browsing habit. The *Mesohippus* evolved from this in the course of 20 million years as is confirmed by the occurrence of fossils of *Mesohippus* from the oligocene rocks of North America. *Mesohippus* was slightly taller almost of the size of a big dog. It had three toes on each foot with the lateral toes still partly functional. From *Mesohippus* evolved

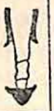
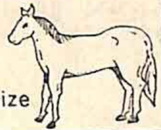
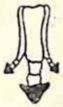
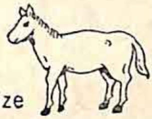
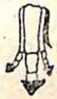
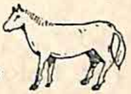
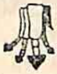
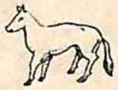
ANIMAL		ANIMAL SIZE
Equus (Recent)	One central toe splints of 2nd and 5th 	Modern size 
Merychippus (Miocene)	Three toes 	Donkey size 
Mesohippus (Oligocene)	Three toes splint of 5th 	Sheep size 
Eohippus (Eocene)	Four toes. 5th undeveloped 	Fox size 

Fig. 25.6. Figure showing table of different horses and their characteristics. In bracket, their probable periods are shown.

Mercyhippus during course of ten million years, i.e., in the Miocene epoch. Almost of the size of modern ass, *Merychippus* had three toes but only the central one was functional. The teeth and jaws were adapted for chewing and grazing. The change from browsing habit to grazing habit was essential for the survival of species because it was the period of appearance of dominant grasslands. *Merychippus* evolved further to give rise to *Pliohippus* which had a single toe on each foot. The fossils of *Pliohippus* have been recorded in the Pliocene epoch. The modern horses, asses and zebras have evolved from *Pliohippus*. The one toed modern horse is *Equus* which

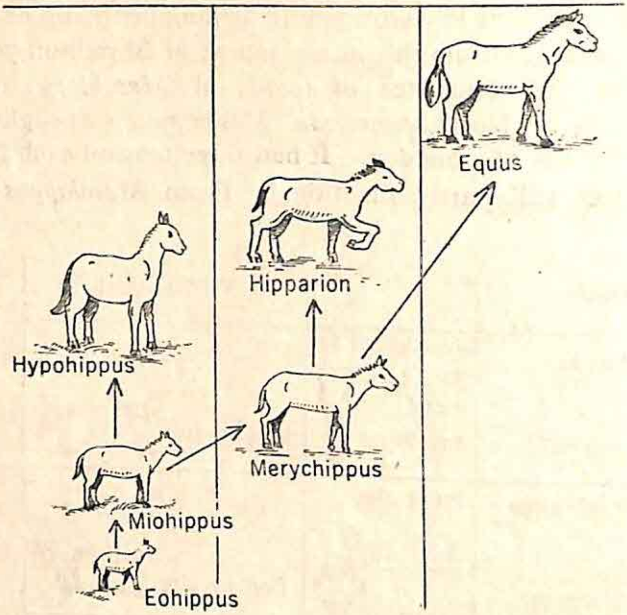


Fig. 25.7. Evolution of horse (Redrawn from Simpson, 1966).

migrated to Africa, Asia and Europe from its birth place, the North America. The history of evolution of horse appears true due to well preserved fossil records of various intermediate forms.

GENERAL QUESTIONS

1. With the help of suitable examples, write a note on that evolution has occurred.

2. Write short notes on

(i) Evolution of modern horses, (iii) *Drchaeopteryx*,
(iii) Embryological evidences on evolution; and (iv) Fossils and evolution.

□ □ □

CHAPTER 26

DIFFERENT THEORIES ON EVOLUTION

The word 'evolution' arising from Latin word *evolvere* (to unroll) means the development of some thing by natural process. Thus when we talk of organic evolution we refer to the evolution of life on this earth. Once evolution is taken for granted, the big question comes in—How did it take place ? or how evolution has taken place ? In answer to this question different theories have been put forth.

THE THEORY OF LAMARCK [Lamarckism]

LAMARCK (1744-1825) : A great French Naturalist and a pioneer evolutionist of pre-Darwinian period, Jean Baptiste Pierre Anotoine Monet Lamarck was born at Bazantin. Picardy, France on 1st August 1744 and died in Paris on December 18, 1825. He was related to an impoverished noble family and his father was a soldier. So was Lamarck also in early period of life. In 1766, he resigned from army as Lieutenant owing to illness, and turned to the study of medicine. In 1744, he published *Recherches sur les causes des principaux faits physiques*" which did not earn anything to his reputation. In 1778, he published *Flore Franchise* which got him admission into *Academic des Sciences* and in 1779, he gained membership of Royal Academy of Sciences. He was appointed to the Chair of Botany in 1788.

Lamarck was imaginative and visionary thinker. His historical contribution *Philosphic Zoologique* was published in a form of book in 1809. Other important publications include *System des animaux sans vertebres* 1801) "Histoire naturelle des animnax sans vertebres" (7 Vols.; 1815-1822) etc. Thereby he established echinoderms, arachnids and crustaceans and propounded the modern classification of animal kingdoms.

Lamarck was married thrice and his wives died before him. He had eight children. From his first wife, there were two daughters, Rosalie and Cornelie, who never married. He died in great poverty and earned no reputation during his life time. In the last decade of his life he became blind and lived with his daughters. They extended earnest services to him till his death. However, his statue was unveiled in Paris, in 1909, which depicts him lost in deep thoughts with a legend on the base "The founder of the theory of evolution". On the back of pedestal, there is a bas-relief showing Lamarck blind and old and besides him, there is a young lady Cornelie expressing devotion to her father. Her emotional and encouraging sentence to her father is also inscribed *Posterity* (future generation) *will admire you, my father; posterity will establish your fame.*

Lamarckism

Lamarck suggested that environment is an important factor for evolutionary changes affecting the shape and organisation of animals. His conclusions may be enumerated in the form of following four laws:

1. INCREASE IN SIZE : Living organisms and their component parts tend continually to increase in size upto a certain limit which comes by itself.

2. ENVIRONMENTAL EFFECT : Production of new organs results from a new need due to changing environment (movement) which this need starts and maintains.

3. EFFECT OF USE AND DISUSE : The development of an organ is proportionate to the use made of this organ. That is to say, if an organ is used constantly, it will tend to become highly developed whereas disuse results in degeneration.

4. INHERITANCE OF ACQUIRED CHARACTERS : The characters which are acquired during course of life due to changing environment are preserved and are transmitted to the new offsprings. These changes are increased by successive additions over a period of time.

Lamarck's work chiefly centres around the inheritance of acquired characters. He suggested that envrionment is an important factor for evolutionary changes affecting the shape and organisations of plants and animals. An organism lives continuously in some kind of surroundings which always work on it. The immediate effects of environment are not always obvious but it takes a long time before the results are manifested. Environment Is also not stationary and it changes. Such changes impose the changed needs by a change in the habits or activities of the organism. The new activities involved greater use of certain organs and lesser of others. Lamarck believed that continuous and extra use strengthens and develops an organ and disuse results its degeneration or even disappearance.

Lamarck enumerated this principle with the example of Giraffe. He considered it originally a deer grazing grass and herbs. When their supply became inadequate, it started feeding on the leaves of the trees. It required greater height to reach the higher leaves. In the process of reaching the only available food from the tall trees, the neck was stretched and thus long neck became its characteristic feature. The result was the modern Giraffe. Almost similar explanation for the evolution of horses was extended by him. He believed that ancestors of modern horse left the woods and took a life on dry plains of West. The change of habitat exercised changes in the length and structure of legs which prompted his increased speed over hard ground. Thus, the new generation began life with better legs adapted for fast running. By continued use of legs for running, the horses of second generation with incereased strength and length were evolved and ultimately modern horse has evolved.

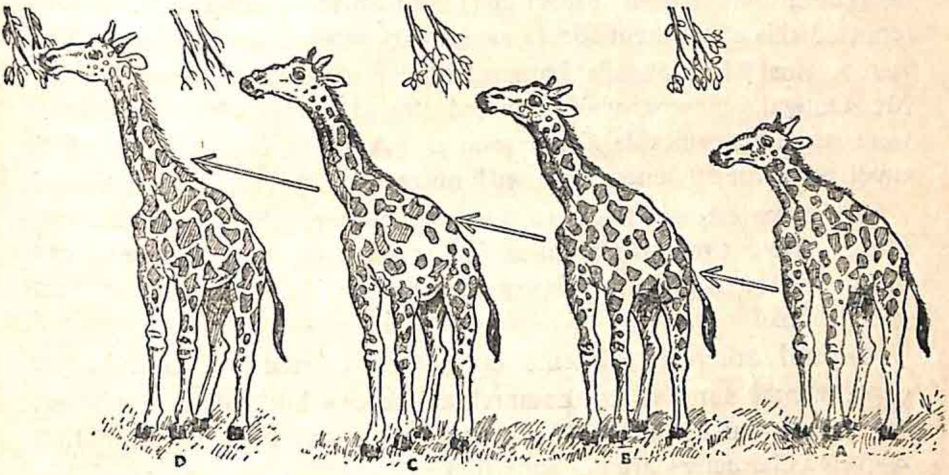


Fig. 26.1. Figure explaining the origin of modern Giraffe.

Demerits of Lamarck's Theory

The Lamarckian theory met great objections particularly against the assumption of the inheritance of acquired characters. In the more recent years, several experiments have been carried out. Paul Kammerer (1907) supported Lamarck based on studies on the effect of coloured surroundings on the skin of colour of spotted salamander, *Salamander maculosa*. But when this experiment was repeated by

Frisch, Przibram, Dembouski etc. the results could not be proved and the effects of surroundings were not transmitted.

Mc Dougal's experiment (1938) on learning in rats gave a single and doubtful exception. Several generations of rats were trained to escape through dark hole from lighted hole where electric shock was given. He found that speed of learning increased from generation to generation. Several objections were raised against him. Crew repeated this experiment for 18 successive generations but his results were against Mc Dougal's Lamarckian claim. Crew suggested that Mc Dougal unconsciously selected strains of rats whose genotypes were specially suitable for training. Agar (1954) repeated this experiment for 50 generations with no result.

The greatest set back to Lamarckism was given by Weismann (1892). He cut the tails of mice for several successive generations but it was found present in last generation as long as that of first generation.

Several other experiments have been carried out to find some experimental support to Lamarckian theory but all were without any substantial favour to it. So, this led to the conclusion that acquired characters are not inherited.

OBJECTIONS : Following are a few important objections to Lamarckism:

1. Lamarckism as such can not be applied to lower organisms where cellular differentiation is obscured.

2. Lamarck's idea of evolution has not been accepted by morphologists.

3. According to Weismann, the new organism develops from the germ cells of parent and not from the somatic cells. The germ cells are not affected by body cells or environment.

4. The conscious efforts as is in giraffe and other animals can hardly be expected in plants.

5. The theory has no experimental proof in favour.

The Lamarckian theory may be now of little importance but his contribution to the discussion of evolutionary problems was great. Carter (1960) wrote that "By putting forward the first theory to explain the cause of evolution, Lamarck at least made biologists realise that the traditional disbelief was not unquestionably right. After Lamarck's time, the possibility that evolution was true, was always at the back of men's mind."

THE THEORY OF DARWIN [Darwinism]
(Darwin's theory of Natural Selection)

Darwin (1800–1882)

Charles Robert Darwin was an English Naturalist and is regarded as a pioneer worker in the field of evolution. He was born at Shrewsbury, Shropshire on Feb. 12, 1800. He first studied in Edinburgh but medicine never appealed him and he joined Christ College, Cambridge and did his graduation. His reputation at Cambridge earned him an appointment to the post of Naturalist in H. M. S. He, then as a Naturalist took part in a voyage of exploration around the world on Beagle in Dec. 1831. Darwin spent about five years in the voyage and visited South America, the Galapagos, Australia, New Zealand, Tasmania, Maldives, Mauritius etc.

Darwin married his cousin Emma Wedgewood in 1839 and was blessed with two daughters and five sons. After voyage, he led a life almost as an invalid. He was acknowledged as a great Naturalist before his death which occured on April 19, 1882. He was burried in Westminster Abbey, next to Sir Issac Neuton.

He has several important and historic publications to his credit, e.g., The Structure and Distribution of Coral Reefs (1842); Volcanic Islands (1844); Geological Observations (1846); On the Movements and Habits of Chimbing Plants (1864); The Variation of Animals and Plants under Domestication (1868); The Descent of Man and Selection in relation to Sex (1871); The expression of emotions in Plants and Animals (1872); Insectivorous Plants (1875); The Different Forms of Flowers on Plants of the same Species (1877); The Power of Movements in Plants (1880) etc.

A theory that has excercised the greatest influence was the theory of natural selection. It was put forward by him in his book *Origin of Species* which was sold out on the day of publication, Nov. 24, 1859. It was regarded to be the most important event in the biological history. He supported his observations on the basis of his collections made over a period of several years. His staunch supporters were T. H. Huxley (England), A. Gray (America) and E. Haeckel (Germany). Almost similar conclusions were drawn by A. R. Wallace.

Darwinism

Darwinism is considered as first scientific explanation of evolution. The main idea of Darwin's theory on evolution may be summarised as follows:

1. OVERPRODUCTION : According to Darwin, every individual increases its race in an indefinite geometric number and this unchecked multiplication would lead to over population of the earth as majority of forms increase rapidly. For example a plant of shepherd's purse produces nearly 40,000 seeds and the evening prime rose 118,000 seeds. If all the offsprings of any species survive and reproduce, the species would occupy all the available space on the earth within next few generations. A single plant of fern produces millions of spores and the enormous multiplication capacity of becteria is well known. A bacterium, which produces two individuals in 30 miniutes, if multiplies continuously and all progenies survive, the number of descendants produced in 24 hours would run into billions.

This fact is also applicable to slow breeders. Elephant is perhaps most slowly breeding animals. It lives about 100 years with an active breeding period of 60 years. A single female would produce six offsprings. If all these survived and multiplied, only in 750 years, the number would reach about 19,000,000. There are many more examples like this.

These data are fantastic as even in the slowest breeding species, its number would soon become much larger than can be supported on this earth. But this does not happen. The number of offsprings that reach maturity and reproduce is just enough to maintain the species, i.e., survivor's number remains relatively constant over long periods of time.

2. STRUGGLE FOR EXISTENCE : It explains the reason for the first law. The disparity between large number of offsprings produced and only a few survived is due to ceaseless struggle that goes on among the descendents of plants and animals. One of the most important limiting factors is the limited food supply. The limited food supply checks the unlimited increase in number of individuals of a given species. So, there is always a struggle. It is a many-angled phenomenon. It is for food, space, light for plants, it is for enemies and predators that kill and destroy, it is for unfavourable environment etc. In general, it is referred as struggle for existence. Darwin explained it in three forms:

(i) Struggle against the forces of nature
(ii) Struggle between the species (interspecific competition)
(iii) Competetion within a species (intraspecific competition).

The struggle against the forces of nature relates to varied conditions of environment such as climate, soil, water etc. Only most suitable forms can survive. Of these three forms, the most intense struggle occurs among the individuals of some species because of identical requirements. The majority of participants are destroyed without producing any offspring. Only small number comes out successful and reaches the stage of reproduction. So the struggle of existence is for the self preservation and self perpetuation.

3. VARIATIONS : In this third fact of the theory he emphasised the problem of appearance of differences between organisms. As there is struggle for existence, there is also variation of living beings. No two living beings are identical and there are differences between the two individuals of the same parents. All these changes are termed as variations.

He further proposed that variations might be continuous or correlated, definite or indefinite, hereditary or nonheriditary. Continuous variation is the one in which the organ liable to be changed in a certain direction keeps on changing in the coming generation. Correlated variation is the one in which one change in the individual's body leads to another change. Definite variations are those which appear in all the members of an entire group of the same under the influence of changed conditions of climate and food. Indefinite variation is accidental. Hereditary variations play role in the origin of new species while non-hereditary are environmental induced and play no part in development of species.

4. SURVIVAL OF FITTEST OR NATURAL SELECTION : In the struggle for existence, the individual which is best suited for the particular environment and which is provided with variable variations will survive and propagate its kind. Darwin called it natural selection while Herbert Spencer called it survival of fittest. He proposed that survival of fittest is a type of selection exercised by natrure in a quiet and imperceptible way. Under this natural selection the individuals which have not undergone variations will not survive. When there is a change in environment, there is a change in character also surviving under natural conditions. The individuals which fail to develop new variations will not fit in the community and some become extinct.

Darwin elucidated the example of Giraffe on the basis of his theory. He is of the opinion that in any generation of Giraffe, the length of neck tend to vary. The individuals that have slightly longer necks than their fellows will get the best food and will survive.

Their number will increase. The environment faces a continual change and therfore evolution will be the continuous process.

Origin of New Species

Darwin was of the opinion that the survival of the fittest will have new structures as well as new factors and modes of behaviour. These characters tend to increase qualitatively from generation to generation until a distinct form known as new species will be formed. It is through these changes that an individual reaches the present state of fitness. According to Darwin, species is the basic taxonomic unit and is variable.

EVIDENCES IN FAVOUR OF DARWINISM : There are a number of evidences which have been put forth to prove Darwinism.

(i) There were a huge number of animals in the olden times but only a few of them persisted due to scarcity of food and changing climate. The animals which persisted had their habit and habitat changed and were able to dominate in that area.

(ii) The production of various domestic varieties by artificial selection is also an evidence.

(iii) In Austria-Hungary, there is a zone of 600 m. which is divided into eight stages and in all these stages a transition from shell-less to shelled mollusca is found.

(iv) Paleontological evidences clearly explain the Darwinism. For example, ancestors of horses were four toed and from them modern horses have originated by reduction of three toes, fibula and ulva.

EXPERIMENTAL EVIDENCES TO DARWINISM : 1. *Weldon's experiment* : Weldon carried out an experiment to find validity of Darwinism on shore scrabs of *Plymouth Sound*. He showed that under the changing enironmental conditions natural selection acts upon minute fluctuating variations of qualitative type so as to produce an alternation of species. The results were in conformity with Darwinian concept.

2. *Poulton and Sander's experiment* : They placed a number of butterflies, pupae under conditions favouring protective coluration and others under opposite conditions. The majority under protective colouration was a real survival factor and also had an advantage in the struggle for existence.

3. *Davenport's experiment* : He took a few chickens which were black, white and checkered in colours and allowed them in the fields. He saw that the hawks killed most of white and black coloured but

spared to a large extent the checkered ones as it got comaflaged (inconspicuous) by the background.

OBJECTIONS TO DARWINISM : Inspite of being universally accepted, Darwin's theory has many objections.

1. The greatest weak point in Darwin's theory is that it does not clearly explain the origin and transmission of variations. Also he did not oppose Lamarck's view of inheritance of acquired charactere. Instead, Darwin stated that the parts of body give off tiny particles called **pangenes**. These are present in the body fluid and collected in eggs and sperms. These, he claimed, are responsible for a particular character in an individual. However, the hypothesis of pangenes is untenable.

2. Weismann is of the opinion that Darwinism is too much based on Lamarckism.

3. According to Darwin the minute fluctuating variations were not inherited while the large fluctuating ones were inherited. He was of the opinion that sports or mutations were too infrequent and did not provide the necessary basis for selection but now it is known that mutations are of much frequent occurrence.

4. Darwin never distinguished between somatic and germinal variations and stated that all were heritable.

5. Darwin's idea that variations happen by chance is objected.

6. This theory does not explain the occurrence of vestigeal organs.

7. Darwin does not explain the phenomenon of mimicry, though it is of elaborate use.

8. Certain characters in an individual undergone over specialisation and have become cause of hinderance. For example, *Irish Elk* has large size of horns. Natural selection should have kept them within the limits of usefulness.

9. Darwin dealt with the quantitative approach of variation whereas the differences between the species are qualitative.

10. The absence of connecting links can not be explained by Darwinism.

11. Natural selection explains the survival but not the arrival of the fittest.

Taking in view the above objections, it is now held that the natural selection is not the only factor in evolution. Darwin himself had said, "Natural selection has been the main but not the exclusive means of modifications." Scientists have come to say that he had not proposed a principle governing evolution but only a means by which its failures are eliminated.

NEO-DARWINISM

Darwinism was the first coined term to denote the type of evolutionary mechanism explained by Charles Darwin as organic changes. Darwinism was largely favoured by the biologists but at the same time there were objections too. Later with more recent researches and knowledge certain ideas were dropped off or certain were added and substituted. Those scientists who take the Darwin's theory in modern light are called Neo-Darwinians and their concept is called Neo-Darwinism. Neo-Darwinsm has obtained support by T. Dobzhansky, G. L. Stebbins, Ernst Mayr, Julian Huxley etc. Carter (1960) described Neo-Darwinism as "modification of Darwin theory taking into account the results of more recent work."

These scientists performed several experiments which removed discrepencies of Darwinism. At that time, the knowledge of cytology and genetics was insignificant. Now the difference between inheritable and non-heritable variations can be explained on the basis of mechanism of inheritance. Genes as the specific determiners and intricate mechanics of chromosomes during cell division have also been discovered. Radiation can now artificially induce mutations. Chemical composition of hereditary substances are also known. The mathematical formulae of Ronald Fischer and Wright have been applied to handle the theoretical genetics of entire population. The modern concept has replaced an individual organism with breeding population or called gene pools. Thus, an individual is an aggregation of many gene combinations and there is a constant variation from generation to generation. Mutations are also produced. Neo-Darwinism also show that variation should be accompanied by isolation. These slight and larger isolations result in the formation of subspecies, species and genera. According to Neo-Darwinism no two individuals are alike, that all groups vary and that some of these variations are inherited to the succeeding generations.

WEISMANN'S THEORY OF GERMPLASM

August Weismann (1834-1914) was a devoted disciple of Darwin and stubborn opponent of Lamarckian theory. He was also not fullest supporter of Darwin's theory and made certain modifications. His modifications made the basis of Neo-Darwinism and opposed the Neo-Lamarckism.

According to Weismann, the variations are of two types. One variation is with which an organism is born and the other which an organism acquires during his life time.

Weismann's theory of germplasm is based on the concept that living organism is made up of two distinct kinds of material—the *germplasm* and the *somatoplasm*. The germplasm is solely responsible for the development of reproductive parts in the living organism. It remains different from the general body of the organism which develops from it. Somatoplasm is the general body, i.e., except the reproductive bodies. Germplasm reproduces and gives rise to the offsprings in which again the germ plasm is separated from new somatoplasm. Thus, it is seen that somatoplasm is newly formed from generation to generation while germplasm is the same. Somatoplasm is discontinuous while germplasm is continuous. In dioecious plants, there is a confluence of maternal and paternal materials in the germplasm stream in each generation.

Weismann concluded that somatoplasm being discontinuous, any variation in it will not be passed on to the next generation, while any change in germplasm is surely inherited. There is no influence on germplasm by the somatoplasm. His statement was in oppostion to Darwin's theory of pangenesis which tried to explain the influencing of germplasm by somatoplasm.

MUTATION THEORY OF HUGO DE VRIES

Hugo de Vries was the first Dutch Botanist (1848-1935) to give his views on origin of species by mutation. He expressed his views that the new species arise not by natural selection of minute fluctuating variation but by sudden variation as mutation. According to de Vries, mutations are heritable and can be useful or injurious. His research work based on a plant called of 'evening prime rose' *Oenothera lamarckiana* growing in a field near Amsterdam. In addition to the normal progeny, plant produced in small numbers a new type which he called mutants. These mutants differed quite a lot from the normal progeny and propagated truly. Recent investigations have shown that mutation does not in itself constitute a complete theory of evolutionary change. The work of de Vries was supported by several other reputed workers such as Gales in England

and Mac Dougal and Shull in America. (The mechanism and other details regarding mutation have been explained in a separate chapter.)

Differences between Variations and Mutations

1. According to de Vries, mutation is the sole material for evolution to take place while Darwin regarded fluctuating variations as the means of evolution.

2. de Vries mutation theory shows that changes are sudden and elementary species are fully formed.

3. Darwin's theory shows that new varieties arise by selection through various variations while mutation theory shows that new varieties arise spontaneously.

4. According to Darwin, the development of varieties into new species is a result of further selection, while mutation theory considers them as a result of appearance of new mutations.

Advantages of Mutation Theory

1. In mutation, elementary species are fully formed from the very beginning and there is no question of incipient stages in the development of an organ.

2. The mutations which are better fitted only survive.

3. The new types formed are different from the parent and have better chances of surival.

4. The offsprings are better adapted to changing environment than the parents.

5. Differences between related species is in the structure of unimportant organs.

6. Certain useless or harmful characters also appear in mutation, they may have adverse effects on the race and still persist.

However the modern concept of evolution is little different and has been discussed separately.

Limitations of Mutations

The previous ignorance that mutations arise spontaneously and unexperimentally for no special reason has been rooted out. Now it is held the mutation though occurring spontaneously have an extremely low but relatively constant rate. Mutation may also arise in a random or haphazard way with having any need to the organism or just uselessly. Some scientists have said that this randomness of mutation cannot provide any constructive matter for the evolutionary changes to take place. Moody (1964) opposes this view and states that this randomness has some limits. Blun (1955) showed that gene mutations are limited by the physical and chemical structure of gene itself.

SYNTHETIC THEORY OF EVOLUTION

It is a collective name to the generally agreed modern approach to resolve the problem of evolution. The theory is an outcome of work done by scientists of modern era. It accomodates mutations as well as natural selection both as important means of evolution. Several other factors which were not visualised by Darwin and de Vries have also been incorporated. These include basic principles of genetics discovered in the present century. It has been widely supported by T. Dobzhansky, R. A. Fisher, J. B. S. Haldane, S. Wright, E. Mayr and G. L. Stebbins.

In a recent book of Stebbins entitled *Processes of Organic Evolution*, the synthetic theory has been discussed in detail. It includes five basic factors involved in the process of evolution—

(i) Gene mutations,
(ii) Changes in chromosome structure and number,
(iii) Genetic recombinations,
(iv) Natural selections,
(v) Reproductive isolations.

Of these, first three are responsible for genetic variations while last two for giving a direction to the evolutionary process. Two other factors have also been included to this respect, namely, migration of individuals and hibridisations. Thus, this theory visualises the role of several factors in the evolution.

GENERAL QUESTIONS

1. With the help of suitable examples discuss the theories on organic evolution.

2. What is Darwinism ? How does it differ from de vries theory of mutation and evolution.

3. Write short notes on :

(i) Neo Darwinism, (ii) Lamarckism,
(iii) Synthetic theory of evolution, (iv) Survival of fittest.

■ ■ ■

CHAPTER 27

PLANT BREEDING

The term plant breeding is referred to the methods used for the improvement in heredity of crops and production of new crop varieties which are better than their parents. This branch, in fact, has contributed maximum to the increase in food production all over the world and is therefore of increasing importance.

AIMS AND OBJECTIVES : The basic aim of the plant breeding has been to produce new crop varieties which are improved than existing types. And according to the choice of the characters required for particular crops, its aim and objective vary. Some of the objectives to produce by this method may be as follows :

(i) higher yield,

(ii) better quality,

(iii) resistance to diseases, insects, pests etc.,

(iv) resistance to frost, flood, drought, wind etc.,

(v) changes in duration of crop maturity (earliness and lateness as needed),

(vi) change in growth habits (such as dwarfness, less branching, tallness, profuse branching etc.),

(vii) winter hardiness,

(viii) response of fertilisers,

(ix) easy thrashability,

(x) adaptability to various climatic regions etc.

Methods of Crop Improvement

The object of plant breeding is achieved by different methods. Some of them are as follows :

1. Selection,
2. Hybridisation,
3. Plant introduction and acclimatisation,
4. Mutation breeding.

The selection method is discussed here while hybridisation and mutation breeding have been discussed separately.

SELECTION

Selection method of crop improvement is prehistoric and not only the man but nature itself has adapted this process (natural selection). It is therefore the oldest method and is quite common even today. It can be further divided into two types :

(i) NATURAL SELECTION : The natural selection is a rule in nature and is the basic cause of evolution. Darwin suggested that in nature, the fittest only survive while rests are wiped out.

(ii) ARTIFICIAL SELECTION : From the mixed population, the plant breeders select certain types of plants for their own advantage. This method is called artificial selection. It may be of three types—

(A) Mass selection
(B) Pure-line selection
(C) Clonal selection

Mass Selection

It has been by far the most common, simplest and oldest method of crop improvement adopted by the farmers who are ignorant about the knowledge of heredity. In this method, the plants superior in desired traits are selected in mass from the population harvesting. The seeds of the selected plants are sown in the following years which are subjected for the repeated selection, ultimately achieving the desired uniformly improved plants. This type of selection is termed as mass selection which requires several years for perfect achievement.

Application and Uses : By mass selection no new genotypes can be induced. It is mere selection of sorting out the suitable types from already existing ones. The presence of different types in the population is found owing to variability or heterozygosity which may be due to cross pollination or any other reason. So mass selection can be applied to any crop showing heterozygosity. It is common in cross pollinated crops while self pollinated crops show poor response. Mass selection in these materials is made to separate new varieties and to maintain its purity where proper supply of seed is not organised.

ADVANTAGES : It is the simplest and the easiest method of crop improvement because it requires no testing of produced variety, no control of pollination for the production of new variety and it is more an art than science. It fulfils the immediate need of farmers for improving wild or local varieties. Besides, it is the basic method of plant breeding as further methods of crop improvement are excercised after it.

Limitations : The limitations of mass selection can be summarised as follows :

1. Selection remains short lived.
2. Variety produced is heterozygous.
3. There is no control over pollination which may produce still more complications.
4. In further generations, the characters segregate which may cause quick deterioration of the varieties.
5. Mass selection is a must to repeat every year of the crop which would mean to keep a continuous vigilance over it.
6. In the process, the attention is paid only to maternal characters during selection while the genes contributed by male parents are not taken into account.
7. Yield of the crop is considerably influenced by environmental fluctuations. Further it becomes difficult to assess the improved quality of crop either due to environmental reason or due to heredity.
8. It is least applicable to self pollinated crops due to poor presence of heterozygosity.

ACHIEVEMENTS : Above all these limitations, this simple method has given many promising results and, by far, the most of the local varieties of crops have been developed by mass selection. Some examples of considerable interest are given here.

Plant's name	*Varieties*
Gossypium hirsutum	Dharwar American, Dodahatti local, Cambodias.
Arachis hypogea	TMV—1, TMV—2, Ground Nut No. 1, Gangapuri, A. H. 14, A. K. 8—11.
Zea mays	T—19, T—41, T—13, Jaunpur, Tinpakhia Darjeeling White Round, Darjeeling White Flat, Darjeeling Yellow Round.
Pennisetum typhoides	T—5, T—55, S. 530, Pusa Moti, R. S. K., R. S. J.
Solanum tuberosum	K—122
Sorghum vulgare	R. S. I. T. 22

Pure-line Selection

Johannsen (1903) suggested the term pure line selection and defined it as 'The descendents of single self fertilised organism of homogenous factorial composition'. Chaudhary (1971) considered the various meaningful definitions and summarised as "A pure line is strain made up of the progeny of a single self fertilised homozygous individual." In pure line selection, two characters—the progeny of single self fertilised plant and genetic uniformity are important. A pure line is composed of many plants all descended from a single self fertilised plant and therefore they are genetically uniform.

What seems more important in the production of pure line is that in self pollinated crops, it occurs naturally and the desirable plants can easily be separated while in cross pollinated crops, the plants are subjected to repeated self fertilisation to obtain the pure lines and to eliminate heterozygosity for making the population homozygous. Such self fertilisation may decrease the vigour and productiveness. However, based on the process of selection there can be several names to pure line selection such as Individual plant selection, Head to row selection, Progeny selection, Pedgree selection, Single line selection and Inbred selection.

The importance of pure line selection is that it is used for production of new varieties and for detection of variation. The pure line selected plants can be used either as new improved variety or as the parents in hybridisation programmes. Since the process is easy in self pollinated crops, the farmers can also execute it for the improvement of crops from yield, stand, maturity, disease and insect resistance etc. points of view.

Some of the important **advantages** of pure line selection may be that :

1. It is the only method to improve the local varieies,
2. It is easier than any hybridisation programme,
3. The varieties are extremely uniform in appearance and performance, and
4. It can be exercised in both self and cross pollinated crops.

Besides, it has several **disadvantages**, e.g.,

(i) It is successful only in self pollinated crops,

(ii) Extra attention and labour are to be paid,

(iii) Sometimes it results in low yielding, albino and dwarf plants in them,

(iv) There is no possibility of introducing new characters, i.e., no new genotypes can be induced through this method, and

(v) Varieties produced are homozygous, therefore, these are susceptible to varying conditions.

ACHIEVEMENTS : Some of the major achievements in establishing the improved new verieties are as follows :

1. *Triticum aestivum* – N.P. 4, N.P. 6, N.P. 12, 9-D, 8 A, K-13 and K-54.

2. *Oryza sativa*— Adt 1, 3, 5 and 10; Co 4, 6, 10, 14; Mtu 1, 3, 6, 9, 13 and Ptb 1, 4, 7, 10. etc.

3. *Gossypium* sp—Co.1, Co. 4, Co 5, A. S. 1543.

4. *Hordeum vulgare*—T. 4., T. 5., R. S. 17.

5. *Arachis hypogea*—T. M. V. 3, T. M. V. 4, H. G. 8, K. T. 18, K. T. 23, K. T. 24.

6. *Brassica campestris*—A.G. H.-A and Y. S. Muzaffarnager.

7. *Corchorus capsularis*—D. 154.

Clone and Clonal Selection

Sugar cane, banana, potato and several other crops are sterile due to non-flowering habits, meiotic irregularities or other genetic causes and produce no seeds. Such plants enforce the use of vegetative parts for their propagation and crop population in them is composed of direct vegetative descendents from different plants. Clones are referred to this concept and it can be defined as "all the progenies of a single plant obtained vegetati vely are known as a clone." Clones are usually characterised by following features :

(i) The clonal progenies are genotypically and phenotypically identical because of their origin and development from the single parent.

(ii) They breed true to parental types and also produce genetically uniform progenies.

(iii) The genotype of clones are often heterozygous but always identical to parents.

(iv) Clones are quite stable and no segregation or variation occurs in them in future generations.

Clones are quite important for certain reasons. They are the only means perpetuating themselves in sterile crops. Occasionally.

these are also used to produce new varieties in vegetatively propagated plants. One of the most important points is that the clones are very useful in preserving the once obtained desirable and superior characters in plants. Hybrid vigours are easily utilised by this process.

The clones are utilised for the improvement of the crop. The most common method is called clonal selection which is the method of selection of desirable clones from the mixed population of vegetatively propagated crops. The vegetative organs that are selected as units differ from crop to crop, e.g., **stem cuttings** are selected in sugarcane, sweet-potato, betel vine and pepper; **tubers** in potato; **suckers** in banana, pine-apple, aloe and agave; **bulbs** in onion and garlics; **corms** in *Colocasia* etc.

There are certain limitations in clonal selection as it is only applicable to vegetatively propagated crops and is unable to create new variations.

The achievements of this methods are not too many. Some clonal selection improved varieties are known in potato such as *kufri red* from Darjeeling Red Round, *kufri safed* from Phulwa; in mango such as *K. O.* 11, *K. O.* 22, *Mundapa Pedda Neelum* and *High Gate* from Gross Michael Banana.

HYBRIDISATION

Hybridisation is more sophisticated and improved method of plant breeding and so far the only effective means of combining together the desirable characters of two or more varieties. Though Cotton Mather (1694) was first man to record natural hybridisation yet Kolreuter (1760) was the first man to use hybridisation for plant improvement. They were followed by large number of workers. Hybridisation may be defined as "the method of producing new crop varieties in which two or more plants of different genetic constitution are crossed together." Five important categories of hybridisation have been recognised.

(i) INTRAVARIETAL HYBRIDISATION : The crosses are made between the plants of same variety.

(ii) INTERVARIETAL HYBRIDISATION : The crosses are made between the plants of two different varieties of the same species. It is also called as intraspecific hybridisation.

(iii) INTERSPECIFIC HYBRIDISATION : When the crosses are made between the two species of the same genes, its called interspecific hybridisation or intrageneric hybridisation.

(iv) INTERGENERIC HYBRIDISATION : The crosses are made between the plants belonging to two different genera. *Raphanobrassica* is the common example of this category.

(v) INTROGRESSIVE HYBRIDISATION : Its behavioural type of hybridisation in which one species is completely replaced by the other after the repeated crossing. Such instance may occur in nature, i.e., when a cross between two species occurs (one of them is found in abundance), the formed hybrids are naturally subjected to back cross with the abundantly occurring species. After some generations, it so happens as a result of back crossing that one species remains while other one is absorbed. It is called introgressive hybridisation.

However, the hybridisation programme of plant improvement is usually followed for three main objectives :

(a) To combine good characters into a single variety.

(b) To increase the range of genetic variability by introducing new recombinations.

(c) To exploit and utilise hybrid vigour.

Procedure of Hybridisation

Hybridisation is a bit technical programme and therefore seeks operation by skilled hands. However, during hybridisation the following points should always be present in the mind :

1. SELECTION OF PARENTS : Such parents are used which can supply the desired important characters and can fulfil objectives of hybridisation plan.

2. SELFING OF PARENTS : The selected parents should be subjected to repeated artificial self pollination in order to eliminate the undesirable characters and obtaining inbreds. The inbreds are always advantageous as they can be easily maintained and can produce better plants after hybridisation.

In the course of artificial self pollination, the seeds of individual plants are harvested separately to isolate different inbreds from mixed population. In cross-pollinated crops, the artificial selfing by different methods is made, e.g., in bajra, mere bagging the panicle to avoid the entrance of foreign pollens, brings out self pollination automatically. In monoecious plants (*Zea mays*) both inflorescence are bagged separately and after the dehiscence of anthers from tassels and emergence of silk from cobs, the bagged female is pollinated with the pollens from the bagged male of the same plant.

3. HYBRIDISATION TECHNIQUE : The inbreds are commonly weak and poor in yield. They are grown in protected atmosphere to avoid insect pests, animals, birds and diseases. The hybridisation technique involves following processes :

(A) EMASCULATION : The emasculation process refers to the removal of stamens from female parent before they burst and shed their pollens. By this process, self fertilisation is prevented. Emasculation is not needed in unisexual flowers but it is quite necessary in the bisexual plants. The method of emasculation varies from crop to crop but the operation is quite simple. Some of the common methods are—

(i) *Forcep or scissors method* : In this process by using forcep or scissors, the stamens are removed by opening the flowers. This technique can be easily adopted in plants with larger flowers, while in small flowers it is equally tedious.

(ii) *Hot or cold water method* : This techninque is being used in plants with minute flowers. The panicles are dipped in hot water at a desired temperature (45°-53°C) for a definite time (1 to 10 mnts). Similarly cold water or alcohol is also used in place of hot water.

(iii) *Male sterile methods* : This technique is in fact not a true emasculation one, rather, it is elimination by using male sterile plants. The cause of male sterility may be cytoplasmic or genetic. However, the male sterility may be induced by certain chemicals such as 2, 4-*D*, *NAA*, Maleic hydrazide and tri-iodobenzoic acid.

(B) BAGGING : After emasculation, both male and female flowers are bagged separately to prevent contamination in staminate flowers and cross pollination in pistillate flowers. The bagging is preferred in the evening as majority of the crops become receptive in the morning. The bags are retained, however, even after the crossing and till the setting of seed is complete.

The bags which are used may be made up of different materials such as paper, butter paper, cloth, plastic, parchment, polyethelene or celophane. The size of the bag depends upon the flower size and for the provision of air exchange some minute pores may be made in the bag, if necessary. The bags are tied at the base with the thread or stiched.

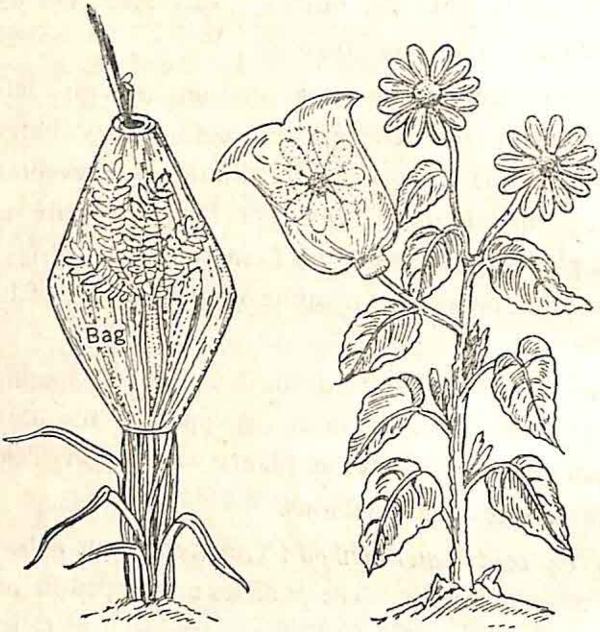


Fig. 27.1. Figure showing bagging in two different plants.

(C) CROSSING : The step crossing is the antithesis of inbreeding. The viable pollens or anther from desired male parents are collected suitably and are then transferred on the stigma of desired emasculated female parent. In wheat, the entire anther is inserted inside the floret with the help of a forcep while in crops like bajra and jowar where the object is quite tedious, both male and female inflorescences are enclosed in one bag in order to facilitate the cross pollination. Any method of transferring the pollen which meets the object can be adopted.

What seems more important in crossing is to evaluate the receptive period of stigma and dehiscence period of pollen. In general case at maturity, the anthers show yellowish powdery mass on their surface while stigma secretes the viscid fluid on its surface.

(D) LABELLING : When the female flower has been pollinated, the following information in a label is immediately tagged for future reference :

Reference no. (Field record no).........

Date of emasculation..................

Date of crossing......................

Details of parents..........................

4. HARVESTING AND RAISING OF F_1 GENERATION : While harvesting, the seeds are handled carefully. The crossed heads are collected separately with their labels. With the onset of next favourable season, F_1 generation (hybrid) is raised from these seeds in protected environment. F_1 is popularly called hybrid variety. Recently, the usage of this term has been restricted for varieties expressing hybrid vigour.

5. HYBRIDISATION METHODS : The F_1 and subsequent generations undergo different selection methods of hybridisation which are different for self and cross pollinated crops. Some of them are described here.

(i) *Pedigree method* : Adopted in the self pollinated crops, pedigree method involves the selection of F_1 population on the basis of desired characters. These are separately sown in the next year to raise F_2. Selection is further made and plants possessing desirable characters are saved, harvested and threshed separately. The seeds of F_5 are utilised in raising F_3 and the process continues upto F_4 and F_5 generations. Now after adequate trials and testings, the seeds are released to the farmers.

However, pedigree method is tedious, complicated and most expensive yet it requires lesser time in producing a new variety. The plants are grown and selected repeatedly upto 7 or 8 generations before these are distributed to farmers. The process suits particularly to those crops where the characters to be combined in the crosses can be easily seen and recognised.

(ii) *Bulk method* : In bulk method, the plants of F_3 generation are not maintained separately rather these are bulked together to form a single F_3 population. Again in F_3, the suitable and desired plants are selected, collected and bulked together. This process is repeated upto sixth generation. F_3 generations are grown separately and comparative trials are being carried out. The best and most promising seeds are distributed to the farmers. Though the system is lengthy yet it is simple, convenient and inexpensive.

(iii) *Back cross method* : Back cross method proposed by Horlan and Pope (1922) is entirely different from both pedigree and bulk method and is applicable to both self and cross pollinated crops. It is successfully used for transferring a single character such as disease, frost and drought resistance. In actual practice, the recurrent or recipient parent (desirable variety) is crossed with an undesirable variety, called donor parent, possessing character which is not present in recipient parent. The F_1 plants are back crossed with recurrent parent and are not permitted to self pollination. It is because of this reason, it is designated as back cross method. The progenies of the back cross, called BC_1, are again back crossed to recurrent parent. This process is continued for several generations upto BC_5 or BC_6. BC_6 plants are permitted to self pollinate to raise homozygous forms in combined characters. Back cross method is quite advantageous as it can be used under any environment. The method is rapid, repeatable, predictable and requires the handling of smaller number of plants. Above all, it does not require evaluation of agronomic performances and back cross derived varieties can be safely released to farmers without evaluating yield and quality. Adaptability of these plants remains well maintained.

However, the most important drawback of the process is that it does not permit the achievement of genic combination from two or more varieties.

(iv) *Composite cross method* : The process involves the simultaneous crossing of several pure lines together. For example, eight parents are selected and are subjected to cross in four groups—$A \times B$; $C \times D$; $E \times F$; $G \times H$. The F_1 of all the four groups of single crosses are subjected to double crosses as—$(A \times B) \times (C \times D)$; $(E \times F) \times (G \times H)$. Finally plants of double crosses (F_1S of double crosses) are crossed with each other to produce the hybrids of all the eight parents. This cross is, that is why, designated as composite cross or multiple cross. The future course of these hybrids may be adopted according to either pedigree or bulk method.

Difficulties and Precautions

Hybridisation programme faces quite many difficulties and requires considerable precautions for its success. Plant breeders always face difficulties in isolation of suitable parents and hybrids. Besides, the plants selected for hybridisation must have the same growing season. If there is any difference in the time of maturity, the programme is bound to fail. To overcome this problem, sowing periods are adjusted in such a way that both male and female parents flower simultaneously. The other difficulty is that during emasculation and crossing, flowers are subjected to injury. If flowers are susceptible to this mutilation, the entire programme may fail.

Legumes are particularly susceptible to injury and that is why hybridisation in them is quite difficult. Above all, one of the commonest difficulty in hybridisation is that F_1 hybrids usually become incompatible and sterile. It becomes the main handicap in wide crosses. The cause of such anomalies are numerous and are related with genetic imbalance.

Achievements

The successful hybrids show improved characters and an overall increase in yield, growth and size can be seen. Hybridisation has added several new qualities to many crops such as wheat (see chapter on Polyploidy), cotton, sugar cane, tobacco, *Brassica juncea*, *B. napens*, cannas, dahlias, roses, poppies etc. The achievements of hybridisation are numerous.

ACHIEVEMENTS OF INTERVARIETAL ORIGIN

1. *Triticum aestivum*	N. P. 165, N. P. 710, N. P. 718, N. P. 783 to 790, N. P. 797, C 281, C 285, R. S. 31-1.
2. *T. dicoccum*	N. P. 201.
3. *T. durum*	Hyb. 38, N. P. 412.
4. *Gossypium arboreum*	Virnar, Maljari, H. 420.
5. *G. herbaceum*	Kalyan, Vijay, Digvijay.
6. *G. hirsutum*	Co 4, Laxmi.
7. *Zea mays*	Ganga Hybrid Maka 1 and 101, Ganga 3, Ganga safed 2, Himalayan 123.
8. *Solanum tuberosum*	Hyb. 9, Hyb. 19, Hybrid 45.
9. *Oryza sativa*	Adt. 20, Adt. 25, S. 1086, S. 1088, S. 1089, T. 136, T. 137. L. 12, P. 502.
10. *Nicotiana rustica*	N. P. 219, N. P. 220, N. P. 222.
11. *Arachis hypogea*	C 501, A. H. 6481, A. H. 6614.
12. *Lycopersicum esculentum*	Pusa Ruby.

ACHIEVEMENTS OF INTERSPECIFIC ORIGIN

1. *Triticum aestivum*	Niphad 4 (Motia × Khapli × N. P. 4).
2. *Gossypium arboreum*	170-Co. 2 or Deviraj (*G. hirsutum* × *G. arboreum*), 134-Co. 2-M or Devitej (*G. hirsutum* × *G. herbaceum*).
3. *G. herbaceum*	I. S. C. 67 (*G. hirsutum* × *G. arboreum*).
4. *Solanum tuberosum*	Hyb. No. 2236, Hyb. No. 1151, Kujri Kuber (*S. curtilobum* × *S. andigenum* × *S. tuberosum*).
5. *Saccharum* sp. (sugar cane)	Co. 312, Co. 313, Co. 419, Co. 421, Co. 449, Co. 527. H. M. 645.
6. *Oryza sativa*	Co. 31, I. J.-1 Adt. 27.
7. *Lycopersicum esculentum*	Pusa Red Plum (*L. esculentum* × *L. pimpinellifolium*).

ACHIEVEMENTS OF INTERGENERIC CROSSES

1. *Saccharum* sp. (sugar cane)	Co. 366. (Sugar cane × Bamboo, Sugar cane × maize, Sugar cane × Sorghum), H. M. 661 = Sugar cane × *Euchlaena maxica*.
2. Grass	Pusa Giant Anjan. (*Cenchrus ciliaris* × *Pennisetum ciliaris*).

N. B. : Mutation breeding has been described under chapter on **Mutation.**

GENERAL QUESTIONS

1. Write a short account on different methods of plant breeding.

2. What is hybridisation ? How this technique has proved beneficial in the improvement of plants ?

■ ■ ■

CHAPTER 28

BREEDING FOR DISEASE RESISTANCE

Agriculture and its allied sciences aim at the maximum production of plants for food, fibre, building material, fuel and some essential drugs, all required for man's necessities, comforts and health. Advances of civilisation, changes in population and its feeding habits bring about changes in crop requirements making agriculture dynamic. Agriculture of present day is the result of continuous progressive researches in the field of various branches of agricultural sciences. Plants being the pivot of our very existance on this planet and anything that interferes with our attempts to grow crop is the point of our major interest.

Along with the gradual change in the agricultural sciences, the attack on plants changed the look and productivity which the man started calling disease. It may however be defined as "a plant disease in a physiological disorder or structural abnormality, i.e., harmful to plant or to any part or products that reduces the economic value." Diseases are produced by the number of organisms such as fungi, bacteria, viruses, mycoplasma etc. These organisms attacking the plants produce a number of fatal diseases. It is estimated that in India, the damage due to the crop diseases runs into several crores of rupees.

Application of Resistant Breeding

To overcome the problem of plant diseases two methods are generally employed : phytopathological or development of resistant varieties. Phytopathological method involves the eradication of the casual organisms, which, in general being done by clean cultivation, crop rotation, application, of chemicals in the form of spraying and dusting, hot water treatment and field sanitation etc. Breeding for resistant varieties involves the principal by which a plant itself can check the invasion of pathogen. Resistant varieties do not need any external agency to fight against the disease causing pathogen, but breeding for disease resistance takes a long time and calls for a high technical skill and intimate knowledge of plant breeding, genetics and plant pathology. However, it is the best process.

Genetics of Breeding Resistance

Disease resistance is a hereditary phenomenon but its uniformity is not certain. In some cases it may be dominant while in other recessive. Disease resistance may be monogenic or polygenic being influenced by a single gene or due to interaction of many genes, respectively. Plant breeders face many problems in this regard. A variety being resistant to one disease is usually susceptible to another disease of the same crop. For example, sugarcane strains resistant to red rot disease are highly susceptible to the leaf rust and whip smut. Similarly rust resistant 'Khapli' wheat strain is highly susceptible to *Helminthosporium* stripe. In other case, it has been found that the cultivated and high yielding varieties are more susceptible to disease infection whereas the inferior and wild varieties are resistant to the same disease. For example, the superior 'Son' variety of banana is highly susceptible to *Fusarium* wilt while the inferior 'Kali' is highly resistant to it. Such problems create hindrances in producing disease resistant varieties with good yield.

SOURCES OF DISEASE RESISTANCE : Disease resistance is much influenced by the heredity than the environment, so it is much attached with gene. The desirable gene for disease resistance may be obtained from two sources. Firstly, it may be from already existing plant material possessing genes for disease resistance and secondly, through mutation by which genes for disease resistance are newly created.

The resistance which is heritable may be present in the cultivated or wild varieties or in the same species of a single variety. It is common in the wild varieties. The resistance found in cultivated varieties is more advantageous because it can be more easily transferred to the other strain through hybridisation. It is very necessary to have a collection of resistant varieties of different crops from all over the world so that these can be used at the time of need. However, when the resistance is negligible in the existing plants or it is present in very low percentage, the resistant varieties are produced through mutation.

METHODS OF BREEDING FOR DISEASE RESISTANCE : There are various methods for the production of disease resistant varieties. Some of them are described here.

1. *Introduction* : In introduction method, the disease resistant varieties are introduced or grown in a particular area where a specific disease occurs in epidemic forms. For example, the early maturing varieties of ground nut (*Arachis hypogea*) from USA introduced in India have successfully conquered the tikka disease (*Cercospora arachidicola*). Practically the introduction method is not so successful as it has got some limitations because of the problems like—

(i) the introduced varieties in most cases fail to acclimatize in the new environment, and

(ii) the introduced varieties possessing resistance for one disease may be susceptible to another disease.

On account of these problems the introduction method can not be a common method.

2. *Selection* : Selection is a better method than introduction and has more chances of producing disease resistant varieties. In this method a particular crop is sown in the field and disease resistant plants are selected. These resistant plants along with the susceptible ones are then grown continuously for many years and the disease resistant varieties are selected. During experimentation, the artificial disease epidemic is created because it is not possible that every year natural epidemic may come. The pathogen is injected or inoculated artificially and optimum conditions of temperature and humidity are maintained. The plants are grown in inoculation tents, green houses and glass houses.

INOCULATION TECHNIQUE FOR SOIL BORNE DISEASES : The soil borne diseases such as wilts, root rots and seedling off are transferred through the soil borne pathogens. In this technique the varieties are grown in the injected soil and if necessary the intensity of disease can be increased by adding more infectious soil from other areas.

INOCULATION TECHNIQUE FOR AIR BORNE DISEASES : In this case the agency of infection is air and artificially it is done by spraying the spores on leaves and plants or by using hypodermic needle to inject a spore suspension into the plants. Common examples of this category are rusts, smuts and spots.

INOCULATION TECHNIQUE FOR SEED BORNE DISEASES : In this case, the technique involves the inoculation of spores to dry seeds. It may also be done by soaking the seeds in spore suspension under vacuum before planting.

After the introduction of disease the plants are grown for subsequent years and the resistant varieties are selected. These resistant varieties are again and again thoroughly checked under different conditions and then only the varieties are recognised as immune or susceptible. This method is also not adequate because the close association found between the resistance and inferior agronomic traits in plants, is the main obstacle which can hardly be overcome by selection in naturally variable material.

3. *Hybridisation* : In hybridisation method, there is a combination of good features of two varieties into one. The resistance from the inferior variety and high yielding from the susceptible variety are mixed and a resistant variety with good yield is produced thus solving the problem.

In hybridisation, back cross and pedigree methods of breeding are usually employed. If the resistant parent is totally unadapted and undesirable, the back cross is used as a breeding procedure. If, on the other hand, the resistant parent is equally good in qualities, the pedigree or bulk method of tackling the segregating generation is used.

BACK CROSS METHOD : This method was first attempted by Briggs (1930) for introducing disease resistance to Wheat and Barley varieties at California. In the process, the recurrent parent is crossed with hybrid progeny in order to get the characters in addition to the disease resistant characters from the non-recurrent parent.

The process is very simple. In this the resistant variety gene is dominant denoted by *RR* and the disease susceptible gene with good agronomical characters is denoted by ***rr***. The former is non-recurrent type while the later recurrent type. The hybrid produced by crossing of the two will be *Rr*, which is subsequently used for back cross generations. *Rr* progenies are back crossed while *rr* progenies are discarded off. The differentiation between *Rr* and *rr* plants can be made by artificially inoculating the pathogen with the plants. *Rr* plants are heterozygous and disease resistant while *rr* are susceptible plants. With each back cross the hybrids acquire more and more agronomic characters of recurrent or recepient parent with the disease resistance from non-recurrent or donor parent. Only the hybrids found disease resistant are back crossed. The disease resistant hybrid from the back cross will be heterozygous (*Rr*) and must be self pollinated for two generations to produce homozygous hybrids, i.e., true breeding disease resistant plants (*RR*).

There is a draw back in the case when the disease resistant character is recessive. The progenies *RR* and *Rr* can not be differentiated and identified. The problem is tackled by back crossing the two progenies with recurrent parent. The progenies are then self-pollinated and the progenies exhibiting heterozygosity for disease resistance are kept and rest are discarded.

In general, the heterozygous progeny is made homozygous to maintain the variety in sexually propagated plants. The number of back crosses to be performed depends on the genetics of disease resistance and the similarity between the genotype of both parents. It is usually done as long as the maximum number of good agronomical characters from the recurrent parent are transferred to the progeny along with one character of disease resistance from the non-recurrent or donor parent. If in case, instead of one, two or more characters are transferred from the donor tot he progeny, separate back crosses are done. Finally all the back crosses are brought together into one line by intercrossing them (convergent improvement).

4. *Grafting* : In case of vegetatively propagated plants the hybridisation process is not possible. In such cases, the disease resistant strains are grafted on the susceptible plants. The method is commonly applied in horticultural crops and fruit trees.

5. *Mutation Breeding* : In case when the already existing gene is absent, the only alternative for producing disease resistant varieties is mutation. Genes for disease resistance are newly created and used in producing disease resistant varieties by mutation process.

Achievements

Indian plant breeders have made untiring arduous efforts in producing disease resistant varieties and success have been achieved in almost every crop under cultivation. Some of them are given here.

Table showing some resistant varieties of important crops.

Crop	*Resistant varieties*	*Disease to which resistant*
Wheat	N.P. 783 & 784	Brown rust
	N.P. 785 & 786	Yellow rust
	N.P. 789	Black rust
	N.P. 809, N. P. 770, P.V 18, W.G. 76, W.G.139, C 307.	All the three rusts of wheat

Crop	*Resistant varieties*	*Disease to which resistant*
Rice	Co. 25 & 26, M. 42	Blast
	Hybrid Kashmere 60	Shedding and blast
	Co. 24.	*Helminthosporium* disease
Sugarcane	Co. 356, Co. 393, Co. 419, Co. 421, Co. 508, Co. 527 K. 30, B.O. 10 & 11.	Red rot
	Co. S. 245, Co. S. 254	Wilt
	Co. 290, Co. 449	Smut
	Co. 214, Co. 315	Mosaic
Cotton	Suyog, Vijai, Kalyan, Virnar, H. 420	Wilt
Groundnut	Kanpur No. 23, M. 20/38	Tikka
Gram	G. 17 and G. 24	Wilt
	G. 1234 and C. 235	Blight
Banana	Basrai	Wilt.

GENERAL QUESTIONS

1. Write a detailed account on breeding for disease resistance.
2. What are the various methods of testing and improving new disease resistant varieties ?
3. Give some detailed account of resistant varieties produced in India.

■ ■ ■

CHAPTER 29

THE HETEROSIS OR HYBRID VIGOUR

The term hybrid vigour or heterosis refers to the increased size, yield, better vegetative growth etc. observed in the F_1 (hybrid) of certain crosses. The value of heterosis was known in the ancient times to breeders as they were producing mules by hybridising two species of *Equus*—the horse and the ass. But the scientific investigation of this event may be traced in short as follows :

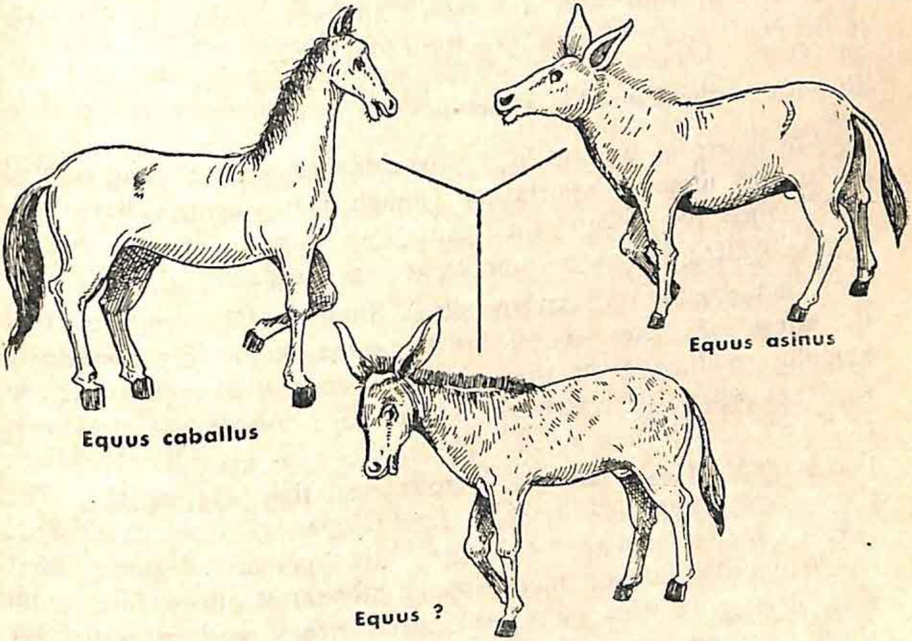


Fig . 29.1. Hybridisation between two species of *Equus* to produce mule.

This phenomenon has been of great interest to both plant and animal breeders. Kolreuter (1763) was probably first to note such an effect while conducting hybridisation experiments in *Nicotiana.* Mendel also observed hybrid vigours in his experiments on pea. However, East, Shull and Hayes studied such examples in details and Shull in 1914 coined the term heterosis for hybrid vigour to make its identity separate from Mendelian inheritance. He stated that "the concept of heterosis is the interpretation of increased vigour, size, fruitfulnses, speed of development, resistance to disease and to insect pests or to climatic vigours of any kind, manifested by cross-bred organisms as compared with corresponding inbreds, as the specific results of unlikeness in the constitution of the uniting parental gametes." In common usage, the heterosis and hybrid vigours are synonymous.

Heterosis may be observed in many characters, e.g., height, size, productiveness, earliness, health, viability, fruitfulness etc. Heterosis occurs as a result of a cross which is swiftly lost if the hybrids are subjected to self crosses. Because of this reason, the seed producing crops require extra care for its maintenance but vegetatively propagated crops such as sugar cane and potato, remain away from such a threat. In these crops, the heterotic property if once produced lasts for a long time.

Theories on the Causes of Hetersosis

Why heterosis occurrs in F_1 hybrids has been a long wanted question of utmost importance. Though many theories have been propounded to explain the cause of heterosis but no one is invariably accepted. A few important ones are described here.

1. HETEROZYGOSITY HYPOTHESIS : Shull (1910) proposed that the effect of heterosis is due to some kind of physiologic stimulus resulting from the union of two unlike gametes, i.e., to say that when like genes are present in a zygote, the stimulus is relatively lesser than when unlike genes are present. It means that genome A_1A_2 would be more efficient than A_1A_1 or A_2A_2. The theory was supported by East and Hayes (1912). Later, East (1936) further enlightened the situation and stated that physiologically active genes exist in a number of allelic forms and when divergent alleles are present together they produce a superior effect in comparison to homologous conditions. This phenomenon is called heterozygosis. The theory has been widely welcomed.

2. GREATER INITIAL CAPITAL HYPOTHESIS : Asby (1930) proposed another theory on cause of heterosis which is based on experimental studies on hybridisation of maize. He visualised that hybrid vigour forms do not differ from their respective parents as regards to cell size, photosynthetic efficiency of leaves or the pattern of sigmoid curve. However, a marked difference is observed in physiological

activities. There is an increased percentage of germination of hybrid seeds. The embryo of hybrid vigour form has a greater initial weight which gives an advantage over normal embryos. This increase is maintained throughout the grand period of growth. The growth is apparantly inherited in a manner of a dominant factor as proposed by Mendel. Because of the greater advantage in embryo size, this theory is refferred as "greater initial capital theory".

This theory was refuted long ago when it was found that the increased embryo size has no permanent significance in corn and peas. East (1936) raised another intelligent objection that an increase in size of endosperm or embryo may itself be one of the manifestations of heterosis.

3. DOMINANT FACTOR HYPOTHESIS : The theory was proposed by Bruce (1910) which illustrates that the heterosis results from bringing together complementary dominant genes from two parents. In such cases, the deleterious effects of the recessive genes are covered by dominant favourable genes. The theory faces several objections. One of them is that heterosis does not show any definite line of segregation in future generations as it should have been if pairs of factors or genes are involved in producing heterosis.

4. LINKED FAVOURABLE DOMINANT GENE HYPOTHESIS : This theory was advanced by Jones (1917). He advocated that heterosis appears due to the complementary action of linked dominant growth favourable genes received from both the parents. Because of linkage, the possibility of absence of any definite line of segregation of heterosis in F_2 can be explained. However, this theory was not experimentally proved.

5. SUPERDOMINANCE HYPOTHESIS : The theory was proposed simultaneously by Fisher aad Hull (1945). Fisher called it by the name 'super dominance' while Hull called 'over dominance'. The theory, however, accomodates the presence of complex of superiority of a heterozygous genotype over an homozygote. Such concept has been emphasised by Jones, Castle, Gustaffson etc. At a sophisticated level, according to this theory, heterosis is the result of interaction of alleles at a single locus. Since there are not many examples of proved over dominance involving a single gene, there is hesitation in accepting the over dominance theory.

6. CYTOPLASMIC NUCLEAR REACTION : Michaelis, Lewis and Shull proposed that the heterotic effect is produced by the physiologic effect resulted out of the interaction of the cytoplasm and the nucleus.

Utilisation of Hybrid Vigour in Crop Improvement

Hybrid vigour has been widely used in breeding inspite of the fact that the very first problem in its proper execution has been to produce hybrid seeds in such a large quantity that F_1 progenies can be grown on a field scale. Besides, in self pollinated crops where heterosis is rare in nature the means of production and maintenance of hybrid vigour have been exploited. For example, by single hand pollination large number of seeds can be produced in crops like tobacco, tomato, egg plant and cucumber. In tobacco and egg plants, nearly 25000 seeds can be produced by single pollination. The method of artificial pollination has been adopted at several places for self pollinated crops. In this reference it is interesting to note that "Pusa purple variety" of brinjal which is regulary distributed to the formers from IARI, New Delhi, is the F_1 seeds of 'Muktakeshi' and 'clustered white varieties'. It gives 40%-50% increased yield over parents.

A few individual examples of achievements are discussed here.

HYBRID CORN : Hybrid corn is by far the best example of the practical utilisation. Shull observed that self pollinated corn (inbreeding) plants show a marked reduction in vigour which could be restored on crossing the inbred lines. He further observed that pure lines (inbred lines) can be established after a few generations by repeated selfing and if these inbred lines are crossed, the hybrids have much greater vigour with uniform productivity. He futher suggested that uniform high yields can be obtained by finding the right pair of inbreds and by repeating the cross. This method however proved very costly. Jones proposed another practical feasible method to adopt. He suggested the crossing of two vigorous hybrid strains and thereby producing double cross seeds. It has been explained in the figure 29.2.

A double cross is a cross of two single cross hybrids, e.g., $(A \times B) \times (C \times D)$. In large scale production of double cross seeds, four rows of F_1 to be used as female followed by a single row of F_1 to be used as male are sown. The female parents are detasseled and are then allowed to be open pollinated by pollen from the single cross hybrid line used as the male parent. It is imperative by this method that male sterile plants can be more successfully used in producing hybrid vigour.

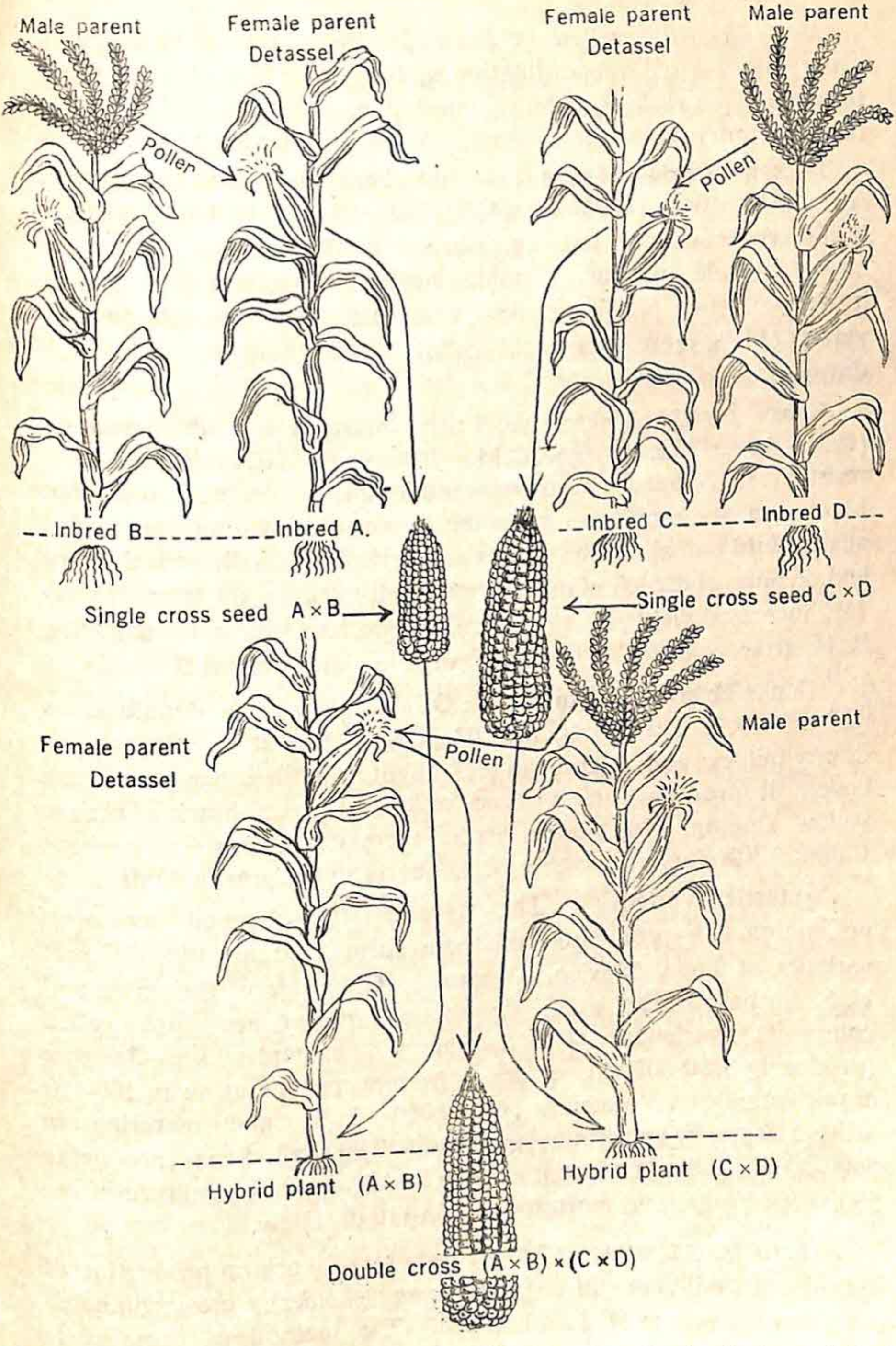


Fig. 29.2. Figure explaining double cross breeding in maize. In the first year inbred plants (*A*, *B*, *C* & *D*) are selected and are cross pollinated in two lines as shown. The female plants are detasseled in the beginning. The F_1 are raised separately and are subject to cross pollination adopting similar method. In this way, in the second year, the double cross hybrid is obtained.

Maize hybrids evolved in India : In India, the maize breeding programme started in coordination by the Indian Council of Agricultural Research with Rockfeller foundation. Some hybrids suitable to our country have been evolved. A few are discussed here.

Deccan hybrid Makka : It has been originated as (C.M. 104 × C.M. 105) × (C.M. 202 × C.M. 201), so it is double cross seed. It is resistant to lodging, downy mildew, rust and leaf blight. While producing bold, hard and bright yellow grains, it develops two big grain-filled ears per plant. Its high yielding variety (145% more than local varieties) that matures in 100 to 110 days.

Ganga Hybrid Makka No. 1 : Its origin is a double cross of (C.M. 100 × C.M. 200) × (C.M. 101 × C.M. 102). While it is resistant to lodging and downy mildew, it is tolerant to top shoot borer and its ears have tight husk cover providing protection against bird damage. The grains are attractive, small, round, hard and yellow and each plant produces well-filled ears. It has nearly 75% increased yield and the plants require 80-90 days for maturity. It is grown preferably in Indogangetic plain and M. P.

Ganga Hybrid Makka No. 3 : Originated from a double cross (C.M. 109 × C.M. 110) × (C.M. 202 × C.M. 111), it is resistant to downy mildew and tolerant to leaf blight. While it has good husk cover, it produces good sized well-filled ears bearing orange yellow, shining, flint grains. It is successfully grown throughout India. With 75% increased yield, the crop matures in 90 days.

Synthetic Varieties : The hybrids are obtained by open pollination. In production of these forms, parents like different varieties of India, Mexico, Venezuela, Cuba, Peru, Columbia and U. S. A. have been used. Synthetic varieties are also called composite varieties. The common examples are—**Jawahar** (producing 5500-6000 kg. of grains/hectare and maturing in 100-110 days), **Amber** (producing 6500-8000 kg/*h*. and maturing in 110-115 days), **Vijay** (producing 5500-6500 kg./ha.), **Sona** (producing 5000-6000 kg./ha. and maturing in 90-100 days), **Kissan** (producing 5500-6500 kg/ha. and maturing in 105-110 days) etc.

HYBRID PEARL MILLET : Larger amount of work on production of hybrid pearl millet or cumbu (*Pennisetum typhoides*), a cross pollinated crop, has been done at Tamil Nadu. The method of breeding is almost similar to that of breeding hybrid corn. Some improved varieties are—

Hybrid X-1 : Involving two inbred lines (P. T. 350×P. T. 348), it is a single cross hybrid which matures in 90 days and gives an yield 17 to 38% more than that of the local variety. It is grown under irrigated conditions.

Hybrid X-2 : Involving two inbred lines (P. T. 1411×P. T. 422), it is a single cross hybrid which matures in 90 days and gives 18 to 47% more yield than local variety. It can be grown both as a rainfed crop and as an irrigated crop.

Hybrid X-3 : Obtained from the cross P. T. 826/7 and P. T. 829/8, this single cross hybrid matures in 85 days and gives 40% more yield than that of any local variety. It can be grown as a rainfed crop and also as an irrigated crop.

Adopting the technique of use of cytoplasmic maal sterility, four hybrid strains of pearl millet have also been produced. In all these four hybrids cytoplasmic male sterility of Tift 23, has been used.

Hybrid Bajara 1 (H. B. 1) : (Male sterile line Tift 23-A×BIL-3). Maturing in 80-85 days, it gives an yield of 2000-2500 kg. of grains/ha. Its suitable to every bajra growing part of India.

Hybrid Bajara 2 (H. B. 2) : (Male sterile line Tift 23-A×J. 115-88). The crop matures in 80 days with an yield about 2500 kg /ha. It is specially suitable to arid and semi-arid regions of Gujrat and Rajasthan.

Hybrid Bajara 3 (H. B. 3.) : (Tift 23-A×J. 104). The variety is resistant to green ear, rust and leaf spot diseases. It matures in 85-90 days with an average yield of grain about 2500-3000 kg./ha. and of straw about 3000-3500 kg./ha. It is specially suitable to low rainfall areas of Gujrat, Maharashtra, Mysore, Rajasthan and U. P.

Hybrid Bajara 4 (H. B. 4) : (Tift 23-A×Kanpur 560). It is resistant to green ear and moderately resistant to smut and blast. Its yield is about 30% more than H. B. 1 and is quite suitable to Andhra Pradesh, Haryana. Madhya Pradesh, Rajasthan, Tamil Nadu and U. P.

GENERAL QUESTIONS

1. What is heterosis ? Discuss its role in the improvement of agriculture.

■ ■ ■

CHAPTER 30

CROP BREEDING IN INDIA

In India extensive work on plant breeding has been done. It was initiated by Dr. B. P. Pal, now regarded as 'Father of Plant Breeding in India'. Now the work has been advanced a lot and following are the important centres of plant breeding—

(i) Indian Agriculture Research Institute, New Delhi
(ii) Central Potato Research Institute, Simla and Darjeeling
(iii) Central Sugarcane-breeding Institute, Coimbatore
(iv) Central Rice Research Institute, Cuttack
(v) Punjab Agricultural University, Ludhiana
(vi) U. P. Agricutlural University, Pantnagar
(vii) C. Z. Azad Agriculture & Techn. University, Kanpur

and several other Agricultural Universities of India.

In the present text following few important crops have been considered along with their recent and standard achievements. Though plant breeding is a tough job, yet India has achieved varieties success in establishing certain outstanding commercial varieties of crops.

WHEAT

Wheat stands as a second major crop of India (first—rice) and its cultivation occupies about 13 million hectares per year. The most common species is the hexaploid wheat, *Triticum aestivum* belonging to the vulgare group and it is grown nearly in 11·5 million hectares. The other two species though grown to lesser extent are tetraploid, *T. durum* and *T. dicoccum*. The people's interest in the advancement of wheat crop can be well realised by recording the increased production from last few years.

The wheat breeding in India was encouraged keeping in view the following aims—

(i) To evolve high yielding varieties.

(ii) To improve the quality of grains suitable to Indian breads (colour, protein content and chapati making quality).

(iii) To increase storage potentialities.

(iv) To evolve disease resistant varieties.

(v) To evolve improved varieties suitable to different climates of India (drought and lodging resistant).

There has been a marked tendency among modern wheat breeders to evolve dwarf varieties which have increased potentialities of yield and resistance to varied atmospheres. All possibilities to improve the wheat crop have been adopted in India. There have been introduction of new varieties of other countries besides mutation breeding, hybridisation and selection. Some of them have already been discussed under chapters 'Mutation' and 'Plant Breeding'.

The importance of dwarf varieties of wheat came into prominence after the discovery of **Norin genes** from Norin-10 strain of Japanese wheat in 1962. These genes are associated with properties like higher yield, disease and drought resistance while maintaining the quality of grains. The credit for such discovery goes to D. A. Vogal and Norman E. Borloug of America. Dr. Borloug was awarded the 'Nobel Prize for Peace', the highest award received by a botanist. Quite a lot of work in India has been done on these lines during recent years. However, some improved varieties are discussed here in short.

Lerma Rojo : A semidwarf Mexican wheat with single dwarf gene. Maintaining higher yield, it is lodging and rust resistant. The grains are small and red coloured.

Sharbati Sonara : It is double dwarf gene variety evolved from Sonara 64 by mutation breeding. Grains are hard and amber coloured. It is resistant to lodging and is quick maturing (120 days).

C. 306 : Parentage—(Regent 1974 × ch 3.3) C. 591 (P 19 × C 281). It has desi Sharbati grains. Recommended for dry regions.

Moti : A three gene dwarf variety, early maturing with white and medium sized grains.

Janak (H. D. 1982) : It is a two gene dwarf variety evolved from a cross between E 557 and H 845.

V. 401 and V. 404 : Both these varieties have medium sized grains and are recommended for northern hilly regions of India.

Kalyan Sona : Parentage— (*Fn*–*K* 58–Nth × N. 108) × Gb. 55. Double gene dwarf, heavy tillering; maturing in 140-145 days, susceptible to brown and yellow rusts; yield 50-60 Q/ha.; Grains—amber coloured, small, semi-hard weighing 32-38 g./1000 grains; chapati and bread making quality good; Ear—oblong, dense, brown colour, smooth, awned, suitable to medium fertility and also to rainfed conditions.

Sonalika (S. 308) : Parentage— [(11-53-388 × An) × (Yt 54 × N. 108)] × L. Rojo. Single gene dwarf; tillering medium; maturity early (125-130 days); rust tolerant; Yield 50-60 Q./ha.; Grain—white, bold, soft, weighing 42-48 g./1000 grains; Chapatis—excellent; Ears—fusi-form, lax, brown, smooth, awned. This variety is highly responsive to fertilisers. It was released by U. P. Agricultural University, Pantnagar.

K-816 : Parentage (Ciano '*S*' × (Sone 64 × Klien Rendidor`. Variety produced at Kanpur; Triple gene dwarf; Good tillering, early in maturity (125-130 days); Resistant to rusts; Yield 45-50 Q/ha. in irrigated and fertilised land; Ear—fusiform, smooth, awned; Grain—amber coloured, semi-hard, lustrous, 34-40 g./1000 grains.

K-68 : Parentage (N.P. 773 × C 13). Tall, medium tillering; Maturity—medium, 135-140 days; Susceptible to rusts; Yield—25-30 Q /ha. in medium fertility conditions; Ear—fusiform, medium lax, blackish awn spreading; Grains—amber, semi-hard, lustrous, 38-46 g./1000 grains. It is most liked variety of U. P. and was released by C. Z. Azad Agricultural University.

K-65 : Parentage (N. P. 773 × C 591). Tall; Tillering—medium; Maturity—medium, 135-140 days; Susceptible to rusts; Yield—15-20 Q./ha. in rainfed conditions; Ear—fusiform, medium lax, hairy blackish awned, spreading; Grains—amber, bold and semi-hard, 42-46 g./1000 grains; Released from Kanpur.

Arjun : (HD 2009). Parentage (L. Rojo 64A × Nai. 60). Double gene dwarf; Tillering—heavy; Maturity—medium, 135-140 days; Resistant to rusts; Yield—55-60 Q./ha. in medium irrigated and fertilised land; Ear—fusiform, lax, awned; Grains—amber, medium size, soft, 32-36 g./1000 grains.

Pratap : (HD 1981). Parentage (E 5557 × HD 845) Double gene dwarf; Tillering good; Maturity early; Tolerant to rusts; Yield·15-30 Q/ha. in low fertility and rainfed conditions; Ear—fusiform, white, glabrous and awned; Grains—amber, medium, bold, semi-hard.

HD 2177 : Parentage [(HD 1962 × E 4870 × K 65) × Kalyan Sona]. Double gene dwarf; Tillering—good; Maturity—medium late; Resistant to rusts; Yield 50-60 Q./ha. under medium fertilised irrigated conditions; Ear—oblong, brown coloured awned, smooth, Grains—amber, bold, hard.

Girija (HS 109 -17) : Parentage (Cajema 60 × E 4717). Double gene dwarf; Tillering good; Maturity early; Tolerant to rusts; yield 40-45 Q./ha. in medium irrigated conditions; Ear—fusiform, medium dense, fusiform; Grains – amber, medium bold, semi-hard.

RICE

(*Oryza sativa*)

Rice is one of the oldest and even today the most popular crop of the world. India, Indo-China and China have been proposed as the centres of origin of Asian Rice. The earliest archaeological remains of rice obtained from India have been dated back to about 2300 BC (Ghosh, 1961), of China to about 2000 BC (Chang, 1968) and of Indo-China to 3500 BC (Gorman, 1971). In modern world, the cultivated rice consists of two species, *Oryza sativa*, the Asian rice cultivated throughout the world, and *O. glaberrima*, the African rice, cultivated on small scale in tropical West Africa.

In India, the rice is grown in all the States and it occupies about 75-80 million acre land. The average yield in the country is 1144 kg./ha. which is far less in comparison to other rice growing countries. Though rice has biologically poor nutritional value to wheat, it is taken by far more people and thus becomes an important food crop. The breeding studies on rice have been looked upon with following objectives.

OBJECTIVES : 1. High yield while maintaining fine cooking quality.

2. Resistance to drought, blast diseases, pests and flood.

3. Resistance to various plysiological conditions such as alkalinity and salinity.

4. Earliness of crop, non-shedding of grains and dormancy of seeds.

5. Good flavour.

BREEDING AND ACHIEVEMENTS : All possible means have been employed to evolve improved varieties at various Research Centres of India, particularly at C. R. R. I. Cuttack. The results from selection, hybridisation and mutation breeding are quite promising. These in part have been discussed under chapters "Plant Breeding" and "Mutation". A few are discussed here.

Basmati 370, Basmati 217, Jhona 349, and Palman 296 are the popular new varieties of northern India. One of the most high yielding variety is EK 70 which yields 3,274 kg./ha. in average. The other high yielding varieties are Jhona 349 (2503 kg./ha.) and Begami 284-14-6-6 (3,211 kg./ha.). The grains of EK 70 are shorter and fine and are therefore less preferred against Basmati. The preference is based largely on grains and cooking quality which varies from region to region in India. Cooking quality is measured by the ratio of amylose to amylopectin and gelatinisation temperature. Although considerable progress has been made in catering to the needs of several parts of the country, requirement of superfine basmati rice for local consumption as well as for export purposes is yet to be met upto the mark. Recently T 23 has been selected from hills to introduce in plains. It is 'basmati' type with an average 12·5 percent higher yield and is early maturing.

Some varieties from Formosa, Iran, Japan, China and Philippines have also been introduced in India. Only a few such as Ac 3400, Ac 3402, Ac 3404 are successful. These are higher yielding than Jhona 349 and Basmati 370. Ac 3400 has shown an increase upto 49 percent over Jhona 349.

A big change in rice breeding came in 1966 when two varieties, the 'Taichung Native 1 (T. N. 1), a Formosa-bred variety and the IR-8, a Philippines variety were released by I. A. R. I., New Delhi. Both these were dwarf, resistant to lodging and high yielding and also were quite suitable to India. T. N. 1 gave an average yield of 43·1g. per plant as compared with 34·6 g. from Jhona 349 and the total yield was 70 maunds/acre. I R-8 proved miraculous and far superior to both T. N. 1 and Jhona 349. This variety gave an yield of about 10,000 kg. or 110 maunds/acre. It has been visualised that if this variety is given proper manure and chemical fertilisers, control of disease and insect pests, its yield may reach upto 150 maunds/acre.

Even with high yields, T. N. 1 and I R-8 have not met the perfect likings of consumers. In market these are sold at cheaper rates. Moreover, a threat of disease susceptibility, problems related to rain and water logging and high expenditure on irrigation and fertilisers have made considerable damage to their reputation among consumers. A new variety 'Jaya' has been released from Banglore which is superior even to I R-8. Like Jaya, two new varieties I E T 1991 and I E T 1039 with superior grain qualities and high yield have been released from Hyderabad. ICAR has also released certain improved varieties for plains. Among them 'Jagannath, Pankaj, Jamuna and Improved Sabarmati' are important.

For Hilly regions of India, the recommended new varieties are Kulu Norin 18, Norin 12, Asahi, Ch. 988 and Ch. 972. Norin 18 was the biggest yielder giving an average yield of 4,683 kg./ha. though it has stiff and coarse grains. T. N. 1 has also been recommended as it has been proved superior to Ch. 988.

Some recently released other varieties are Varangal 487, Taivan 3, Taichung 65, Padma, Nagina 22, Sudha, N. S. J. 98, K-22, T-136, T. 9, Keresail, IR-22, Ratna, Cauvery, Bala, N.C. 1281, I.R. 20, Mtu. 1 etc. A few blast resistant varieties are Co. 25, Co. 26, Hybrid Kashmere 60, M. 42 etc. Var. S. R. 26 B is recommended for salt rich areas. Dudhalichi. Jaisuria, Chakia 59 etc. are flood resistant varieties.

Few varieties suitable to South India are—

Adt 27—Parentage (*Japonica*, Norin 8 × *indica*, GEB 24). Early maturing (105 days), yield 5,200 kg./ha., grains—medium fine, white and good in cooking.

Por. 1— Parentage (Strain Mt u. 1 of Andhra Pradesh × SR. 26-B of Orissa). Suitable to alkaline areas of Tamil Nadu, yield 3,000 to 3,500 kg./ha. grains are finer.

Co. 31—Parentage (*O. sativa* GEB 24 × *O. perennis*). Drought resistant, maturing in 145 days, Grains medium fine with excellent cooking quality.

Co. 25—Parentage (Adt 10 × Co. 4). Long duration variety maturing in 195 days, yield 3,500-4,000 kg./ha., grains white with good cooking quality, blast resistant,

Co. 29—Parentage (Co. 13 × Co. 4). Early maturing variety (110 days), yield—3,500 kg./ha., grains white with good cooking quality, blast resistant.

Co. 30—Parentage (GEB 24 × Co. 4). Variety is of medium duration maturity (165 days), yield 4,000 kg./ha., grains white and good cooking quality, blast resistant.

POTATO

(*Solanum tuberosum*)

The potato is considered as a 'poor man crop'. The Spaniards discovered potato in the 16th century not as a wild plant but as a cultivated staple food used by South American Indians in Southern Chile. Once it reached to Spain, it spread to all poor countries of Europe like Itay, Ireland, Scotland etc. It was introduced in India in the beginning of 17th century by Portuguese businessmen. Now it is one of the most important food crops of India and is grown nearly in 4 lakh hectares area (3½ lakhs in plains and ½ lakh in hills). In U. P., potato occupies a great importance as it occupies 38·6% of total area under potatoes in the country. Farrukhabad district alone occupies 14% of the total area in U. P. But its yield per hectare in U. P. is comparatively poor. It is only about 8 tons per hectare as compared to 24 tons in Gujrat, 16·7 tons in Haryana, 13 tons in Punjab, 12·5 tons in W. Bengal.

The food value of potato in relation to cereals like wheat and rice fetches considerable importance. The amount of carbohydrate per hectare produced by wheat averages 519 kg., by rice 710 kg., and by potatoes from the same land 1335 kgs. Wheat produces protein per hectare about 81 kgs., rice 66 kgs and potato 139 kgs. Similarly the total energy available to the consumer in calories from wheat is 2,422, from rice 3,163 and from potato 5,664, the maximum per hectare. From 100 g. of potatoes with 25% loss in pealing are obtained 12g. starch, 0·23 g. total nitrogen, 0·04 g. fat, 300 mg. K., 35 mg. P., 9 mg. Ca, 0·65 mg. Fe, 0·0075 mg. Vit. A, 0·055 mg. Vit. B_1, 0·008 mg. riboflavin, ·3 mg. nicotinic acid and 15 mg. Vit. C.

The term potato is referred to the tuber of *Solanum tuberosum*. The chief centre of potato breeding is the C. P. R. I. Station, Simla. where under the guidance of Dr. Puskar Nath, the great work has been done.

OBJECTIVES AND AIMS : The breeding of potato has been carried out with following objectives—

1. To evolve high yielding varieties while maintaining good qualities.
2. To evolve disease resistant varieties.
3. To evolve economic varieties suitable to different photoperiods, climates and soil conditions.
4. To evolve attractive size, shape and taste of tubers.
5. To evolve varieties which can be suitably stored.

BREEDING AND ACHIEVEMENTS : For evolving these varieties, techniques like—introduction, selection, hybridisation etc. have been adopted in India. In hybridisation, there are certain difficulties such as some varieties do not flower due to photoperiods and in some flower buds are dropped off before opening. In still other cases, either pollens are sterile or they show pollen stigma incompatibility. However, solving these problems several new varieties have been produced.

1. INTRODUCED VARIETIES : **Phulwa**—It was obtained by selection and was introduced in India by European countries. Its tubers are

small, white and smooth with yellow coloured good quality flesh. It is most popular.

Craig's Defiance : It was introduced from U. K. in 1953 and has been found suitable for hilly regions and Punjab. Tubers are large, long, smooth, white and white flesh.

2. SELECTION VARIETIES : **Darjeeling Red Round** : Its a selection clone with wide adaptability. Its tubers are round and medium sized and has good storage quality. It is one of the most popular varieties.

Kufri Red : Its a selection clone from Darjeeling Red Round and was released in 1957. The tubers are smooth, red and round with white flesh. It has food storage quality. The variety is specially suitable to the plains of U. P., Bihar and West Bengal.

Kufri Safed : Its a high yielding selection from Phulwa. Tubers are medium sized, smooth and white. It has a good storage quality. The variety shows excellent results in the plains of U. P., M. P., Delhi, Punjab and Haryana.

Gola : Its an early maturing selection variety. Tubers are white, pear-shaped with deeply set eyes. Its flesh is white.

3. HYBRID VARIETIES : **Kufri Kuber (O. N. 2236)** : Parentage (*S. cartilobium* × *S. andigeneum* × *S. tuberosum*). Its an early and high yielding variety with medium sized, oval smooth tubers containing white flesh. It is good for cultivation both in hills and plains of Himachal Pradesh, Punjab, Maharasthra and Bihar.

Kufri Kisan (P. S. 454) : Its a single hybrid (up-to-date × O. N. 209) variety suited to long day conditions where it gives high yield. The tubers are irregular, white and medium or large-sized with deeply set pink eyes and white flesh.

Kufri Kundan (Hybrid-8) : The variety has been obtained by cross of Ikseraju and Catadine at C. P. R. I. Simla in 1958. Though it has good performance under day conditions, it gives best results in wet soils of hills. Tubers are medium sized, smooth surfaced, flat-eyed. Its flesh is of yellow colour and therby quite popular. It has excellent storage quality.

Kufri Sakti (C-1) : Parentage (Kufri Red × Kufri Kundan). Its a high yielding variety suited to both hills and plains but is blight susceptible. The tubers are white, medium sized, smooth and round with white flesh.

Kufri Sinduri (C-140) : Parentage (Kufri Red × Kufri Kundan and selection). Its a high yielding and frost resistant variety. The tubers are red, medium sized with fleet eyes. Storage quality is medium.

Kufri Chamatkar (**O. N. 1202**) : Parentage (Ikseraju × Phulwa). Its a high yielding variety better than Phulwa and is suitable for plains. Its less susceptible to viruses but more to late blight. The tubers are medum sized, round with white skin and shallow set eyes. The flesh is light yellow.

Kufri Chandramukhi (A. 2708) : Parentage (S. 4485 × Kufri Kuber). Its high yielding frost resistant variety that matures in two and half months.

Kufri Dewa : Its a new variety (released in 1973) evolved by the combined efforts of CPRI, Simla and U. P. Agricultural University, Pantnagar. Its early maturing (70 days), high yielding (more than Kufri Sinduri) and frost tolerant variety suitable for both hills and plains. It has an excellent storage quality and can be stored for 7 months in shade at room temperature.

There are several other varieties. Few important ones are Kufri Jyoti, Kufri Sheetman (C. 3745), Kufri Yeshwant, Kufri Naveen, Naaga, Kufri Khasigarh etc.

SUGAR CANE

(*Saccharum officinarum*)

Sugar cane serves as the main source of sugar in India and therefore it is considered as a 'cash crop'. The total area under sugar cane cultivation is close to 5 million acres of which 3 million acres are in U. P. alone. In India, the main objectives for the purpose of sugarcane breeding have been—

(i) to evolve high yielding varieties,
(ii) to increase sugar content in the juice,
(iii) to induce delayed flowering,
(iv) to evolve disease resistance varieties,
(v) to evolve varieties suitable to varied climates of India.

The main sugar cane breeding centre of the country is at Coimbatore from where large number of varieties have been released. A few of them are described here.

Co. 419 : Parentage (POJ. 2878 × Co. 290) which has in its composition the complements of *S. officinarum*, *S. barberi* and *S. spontaneum*. The variety is most popular throughout the country and grows successfully in all kinds of soils. On an average, it gives

150 tonnes per hectare with sucrose content 19 percent. However it is susceptible to smuts.

Co. 449 : Parentage (POJ. 2878 × Co. 331) and has complements of *S. officinarum*, *S. barberi* and *S. spontaneum*. Its a high yielding variety and resistant to smut. It has a sucrose content of 18 percent.

Co. 527 : Parentage (Co. 349 × Co. 312 with complements of *S. officinarum*, *S. barberi* and *S. spontaneum*. Its high yielding variety than Co. 449 with sugar content 20% in the juice. It is smut resistant.

Co. 658 : Parentage (Co. 605 × Co. 443). Its a high yielding smut resistant variety.

Co 205 : It is derived from a cross between the cultivated variety 'Vellai' (*S. officinarum*) and a clone from Coimbtore of the wild species, *S. spontaneum*. Its a simple high yielding commercial variety.

Co. 244 : Parentage (Co. 205 × POJ. 213). Its a high yielding variety.

Sugar cane is the vegetatively propagated crop in which both intraspecific and interspecific hybridisation have yielded many valuable results. Most of the important varieties are derived from the crosses involving the cultivated species, *S. officinarum* and *S. barberi* and the wild species *S. spontaneum* This fact can be substantiated on the basis of different varieties. Interestingly, the back cross method of breeding has also been utilised in the production of new strains.

S. officinarum, also called 'noble cane', is susceptible to pests and diseases though its high yielding with high sugar content. *S. spontaneum*, the wild cane, is hardy and resisitant to pests and diseases but has low sugar content. On their hybridisation, F_1 produced were hardy and vigorous with low sugar content. The F_1 was subjected to back cross with *S. officinarum* twice. The progeny from the second back cross has the desirable features, i.e., high sugar content and resistance to pests and diseases. Such a cross is called 'nobilisation'.

In general, some other important varieties are Co. 213 (POJ × Co. 291), Co. 295 (Co. 213 × POJ. 1410), Co. 300 (Co. 213 × POJ. 1410), Co. 356 (*Sorghum* × sugar cane), Co. L. 29 (early variety), Co. 975 (frost resistance), Co. 1148 (P. 4383 × Co. 301)—all round high yielding and late maturing variety, Co. 975 (high yielding, mid-season variety) etc. Some frost resistant varieties are—Co. 1328, Co. 975, Co. 1330 and red rot resistant are Co. L. 29, Co. 1244. Some smut resistant varieties are Co. 290, Co. 449 and Co. 617.

PEARL MILLET AND MAIZE

Both are described under the chapter 'Heterosis'.

COTTON

(*Gossypium* spp.)

In India, four species of cotton are cultivated, *G. hirsutum* (long staple American cotton), *G. barbadense* (long staple Egyptian cotton), *G. herbaceum* and *G. arboreum* (short staple deshi cotton). The former two species are tetraploid while the other two are diploid. Nearly two crore acres of Indian land are under cotton cultivation, the largest in the world. But the production is very low so much so that import becomes essential. Now-a-days, I.C.A.R. is helping a lot to improve cotton crop in India and the main objectives behind the cotton breeding has been

(i) to evolve varieties to produce long and fine type of staples.

(ii) to evolve varieties with high ginning percentage and better spinning quality.

(iii) to produce disease resistant varieties.

(iv) to produce varieties suitable to grow under all varied climates and conditions.

(v) to evolve early maturing varieties to avoid frost injuries.

The cotton breeding is carried out at Bombay, Punjab, Coimbtore and New Dehi. Some important varieties are—

H. 14 : (high yielding), H. 183 (higher than H. 14 with fibre length upto 27·3 mm.), H. 162 (higher than H. 14 with fibre length upto 29·1 mm.) H. 138-13 (sympodial, uniform maturity, early maturing, long fibres), J. 34 (evolved from 45-F × LSS, high yielding. superior fibres, resistant to jassid) etc. These are improved varieties of long staple cotton. However, the improved varieties of desi cotton are—231*R*, G_1 (selection from Ganganagar 1, harsh stapled, short statured, strongly sympodial, high yielding, 12·3 percent more than 231*R*), G 27 (harsh stapled, short statured, suitable for cultivation, high yielding more than 231 *R*, red pigmented, fairly tolerant to water logging and other agroclimatic conditions, resistant to pink ballworm attack), *G* 67 (a reselection form *C* 250, early maturing, high yielding, 28 percent more than 231 *R*) etc.

Some hybrid improved varieties are—Deviraj (*G. hirsutum* × *G. arboreum*), Devitej (*G. hirsutum* × *G. herbaceum*), I.S.C. 67 (*G. hirsutum* × *G. arboreum*), Jayawant, Jayadhar, Co. 4 (Co. 2 × A. 12), Laxmi, H. 14, K. 2, K. 5, Co. 2, Pusa Egyptian and P.R. S. 72 etc. A few disease resistant varieties are—Suyog, Vijay, Kalyan, Virnar, Jarila, H. 420 etc.

Considering the cotton cultivation, India has been divided into four major regions.

NORTHERN REGION : It includes Punjab, Rajasthan and U. P. where *G. hirsutum* and *G. arboreum* are cultivated. In this region the recent improved varieties under cultivation are—Indore 1, 215 *F*, Ganga nagar, 320 *F*, LL 54, H 14231 *R*., Raniben etc.

CENTRAL REGION : The region including Gujrat, Maharashtra, M. P. has three species—*G. herbaceum*, *G. hirsutum*, *G. arboreum* are under cultivation. The newly introduced recent improved varieties are—Daulat, Digvijai, Kalyan, Devi Raj, Vinar, Gaorani 22, G. 46, C. J. 73 etc.

SOUTHERN REGION : It includes A. P., Mysore and Tamil Nadu where all the four varieties are under cultivation. The improved varieties under cultivation are—Parbhani-American, K 6, K 7, Jayadhar, Laxmi, *MCU* 2-3. *N* 14, Nandium, Adonicum, Andrews etc.

EASTERN REGION : Its an almost neglected region from cotton cultivation point of view owing to climatic and soil factors. Only one species *G. arboreum* is grown and the improved variety is D. 46-2-1.

* * *

APPENDIX

SCIENTISTS' CONTRIBUTION

DR. B. P. PAL

Dr. Pal worked eariler on Burmese Charophyta for his M. Sc. degree at Rangoon University. He went to Cambridge for higher training in wheat breeding under Sir R. Biffen and Sir F. Engledow. For Ph. D. thesis he worked on hybrid vigour on wheat. On return to Rangoon, he was appointed as Assistant Rice Reserch Officer at C. R. R. Station, Hmawbi. A few months later he joined Imperial Agricultural Research Institute, Pusa (30th Oct. 1933) where he was to work in collaboration with Prof. K. C. Mehta of Agra. Here he evolved NP. 809 variety of wheat which for the first time had an appreciable degree of resistance to all the three rusts found in India. It was also resistant to loose smut disease. The variety was evolved out of arduous efforts of 18 years.

In 1950, he was appointed as Director of Indian Agricultural Research Institute. Under his regime, the Institute developed enormously and new facilities for gamma radiation and electron microscopy were acquired.

He visited foreign countries several times and attended International meetings. He was elected as Vice President at the International Genetics Congress in Tokyo, 1968. Finally he was appointed as Director-General of Council of Agricultural Research (ICAR). He was chosen President of Golden Jubilee Session of Indian Botanical Society, 1970.

He has also worked on sterility in potatoes and recorded that in some diploid species of *Solanum* self and cross incompatibility is controlled by factors belonging to the same allelomorphic series. He obtained several recessive mutants in chilli and could successfully induce polyploids. Natural tetraploids and triploids were also reported by him. He obtained F_1 heterosis in studies of F_1, F_2, F_3 and back cross of *Lycopersicon lycopersicon* × *L. pimpinellifolium*. He has many valuable review articles to his credit. He made a critical estimate of the data on hybridisation in gram, *Sesamum*, chilli and maize and noted the maximum suitability of maize for hybrid work.

Reviews like "Studies in hybrid vigour; Some aspects of wheat improvement in India; Plant Breeding; Plant Breeding and Genetics, IARI, New Delhi; Recent advances in the technique of plant breeding and seed multiplication and the Scope of increasing the yield of crops under Indian conditions" Speak greatly about his contribution to both—genetics and plant breeding in India.

DR. M. S. SWAMINATHAN

Monkombu Sambasivan Swaminathan was born in Kumbakonam, Madras state, on August 7, 1925. He was educated at Madras Agriculture College, Coimbatore (B. Sc. Ag.) and I. A. R. I., New Delhi. He extended his studies at Agricultural University, Wageningen, the Netherlands, University of Cambridge, U. K. (Ph. D.) and University of Wisconsin, U. S. A.

He is known internationaly for his meritorious contribution in radiation genetics. "The Swaminathan School of Radiation Genetics" is widely known for the pioneer work on the use of atomic energy in crop improvement. His contribution that radiation of food and culture media leads to the generation of radio-mimetic compounds has attracted the attention of radiation food technologists all over the world. He demonstrated that the colour of wheat grain can be readily changed from red to amber and that the quality and quantity of proteins of wheat and rice can be improved by mutational manipulation. Some improved varieties of wheat—Sharbati and Sonara are the result of his arduous work and induced mutation. Using the 'norin genes' for dwarfing, he developed dwarf, non-lodging spring wheat from Mexico wheat under breeding programme. The development of such a wheat has led to the opening of new vistas in wheat production. Besides, he has been active in other fields also. He successfully made a cross between *Corchorus olitorius* and *C. Capsularis* through reciprocal grafting and pollen irradiation. It opened a new era in jute breeding.

He has played a leading role in several extension programmes and is responsible for the organisation of the National Demonstration Programme and for the development of the Jawahar Jounti Seed village. It has been described as major breakthrough in the rapid transfer of information from the scientists to the former.

He owes several reputed awards to his credit for his extra-ordinary and meritorious contribution to biological sciences, e.g.,

1. Shanti Swarup Bhatnagar Memorial Award (1961)
2. Mendel Memorial Medal of Czechoslovak Academy of Sciences (1965). He was the only Asian recepient of the medal
3. Timirazev Academy medal
4. The Indian Journal of Genetics medal
5. Birbal Sahani medal of Indian Botanical Society
6. 'Padma Shri' Award (Jan. 26, 1967)

He was first Indian elected as a vice president of International Congress of Genetics, 1963, Hague. He is a Fellow of National Institute of sciences of India and Indian Academy of Sciences. He has published several scientific monographs and over 160 scientific papers in reputed journals.

■ ■ ■

SELECTED READINGS

ABUEIO, J. G. and D. E. MOORE. 1969. The human chromosomes. Electron microscopic observations on chromatin fibre organisation. J. Cell Biol *41* : 73.

ADELBERG, E. A. 1966. Papers on Bacterial Genetics. Boston : Little, Brown.

ATTARDI, G. and F. AMALDI. 1970. Structure and synthesis of *r* RNA. Ann. Rev. Biochem. *39* :183.

AUERBACH, C. and B. J. KILBEY. 1971. Mutation in eukaryotes. Ann. Rev. Genetics *5* : 163.

AVERY, O. T., C. M. MacLEOD, and M. McCARTY. 1944. Studies on chemical nature of the substance inducing transformation of pneumococcal types. J. Exp. Med. *79* : 137.

BEADLE, G. W. and B. EPHRUSSI. 1937. Developments of eye colours in *Drosophila* : diffusable substances and their interrelations. Genetics *22* : 76.

———and E. L. TATUM. 1941. Genetic control of biochemical reactions in *Neurospora*. Proc. Natl. Acad. Sci. U. S. 27 : 499.

———and———. 1945. *Neurospora* : II. Methods of producing and detecting mutations concerned with nutritional requirements. Amer. J. Bot. *32* : 678.

BEERMAN, W. (ed.). 1972. Developmental studies on Giant chromosomes. New York : Springer Verlag.

BENZER, S. 1955. Fine structure of genetic region in Bacteriophage. Proc. Natl. Acad. Sci. U. S. *41* : 344.

BIRNSTEIL, M. L., M. I. H. CHIPCHASE and B. B. HYDE. 1966. The nucleolus as a source of ribosomes. Biochem. Biophys. Acta. *76* : 454.

BLIXT, S. 1975. Why didn't Gregor Mendel find linkage ? Nature. *256* : 206.

BRAUN, W. 1965. Bacterial Genetics. Philadelphia : Saunders.

BRAWERMAN, G. 1974. Eukaryotic *m*RNA. Ann. Rev. Biochem. *43* :621.

BRIDGES, C. B. 1914. Direct proof through non-disjunction that the sex-linked genes are borne by the X-chromosome. Science. *40* : 107.

———. 1916. Non-disjunction as proof of the chromosome theory of heredity. Genetics. *1* : 1, 107.

BRITTEN, R. J. and E. H. DAVIDSON, 1969. Gene regulation for higher cells : a theory. Science. *165* : 349.

BROWN, S. W. 1966. Heterochromatin. Science *151* : 417.

BROWN, W. V. 1972. Text-book of Cytogenetics. St. Louis : Mosby.

CAIRNS, J. 1963. The bacterial chromosome and its manner of replication as seen by autoradiography. J. Molee. Biol. *6* : 208.

CALLAN, H. G. 1967. The organisation of genentic unit in chromosomes. J. Cell. Sci. *2* : 1.

CALVO, J. M. and G. R. FINK. 1971. Regulation of biosynthetic pathway in bacteria and fungi. Ann. Rev. Biochem. *40* : 943.

CAMPBELL, A. 1962. Episomes. Adv. Genetics *11* : 101.

———, 1969. Episomes. New York : Harper and Row.

CARLSON, E. A. 1966. The Gene : A critical history. Philadelphia : Saunders.

CHANDRASE KHARAN, S. N. and S. V. PARTHASARATHY. 1965. Cytogenetics and Plant breeding. P. Varadachary and Co., Madras, 2nd edition (Revised).

CLARK, R. J. and FELSENFELD, G. 1971. Structure of chromatin. Nature New Biol. *229* : 101.

CLEVER, V. 1968. Regulation of chromosome functions. Ann. Rev. Genetics *2* : 11.

CORWIN, H. O. and J. B. JENKINS, 1975. The Foundations of Modern Genetics. Boston : Houghton Miffin.

CREW, F. A. E. 1966. The Foundations of Genetics. New York : Pergamon.

CRICK, F. H. C. 1958. On protein synthesis. Symp. Soc. Exp. Biol. *12* : 138.

———, 1966. Codon-anticodon pairing : the wobble hypothesis. J. Molec. Biol. *19* : 548.

———, 1967. The Croonian Lecture, 1966 : the genetic code. Proc. Roy. Soc. B. *167* : 331.

———, 1971. General model for the chromosomes of higher organisms. Nature. *234* : 25.

——— and L. BARNETT, S. BRENNER and R. J. WATTS-TOBIN. 1971. General nature of the genetic code for proteins. Nature. *192* : 1227.

——— and J. S. GRIFFEN and L. F. ORGEL. 1957. Codes without commas. Proc. Natl. Acad. Sci. U. S. *43* : 416.

DAVIES J. and F. Jacob. 1968. Genetic mapping of the regulator and operator genes of the *lac* operon. J. Molec. Biol. *36* : 413.

——— and M. NOMURA. 1972. The genetics of bacterial ribosomes. Ann. Rev. Genetics. *6* : 203.

DRAKE, J. W. 1970. The Molecular basis of Mutation. San Francisco : Holden-Day.

DUNN, L. C. 1965. A short history of Genetics. New York : McGraw-Hill.

DUPRAW, E. J. 1969. Cell and Molecular Biology. Acad. Press, New York.

EHRLICH, P. R., R. W. HOLM and D. R. PARNELL. 1974. The Process of Evolution. New York : Mc Graw-Hill.

EPSTEIN, W. and J. BECKWITH. 1968. Regulation of gene expression. Ann. Rev. Biochem. *37* : 411.

FOX, S. W. 1964. The Origin of Prebiological Systems. Academic Press. N. Y.

GALINAT, W. C. 1971. The origin of maize. Ann. Rev. Genetics 5 : 447.

GARBER, E. D. 1972. Cytogenetics : An Introduction. New York : Mc Graw-Hill.

GARDNER, E. J. 1975. Principles of Genetics. 5th Ed. New York, London and Sydney : John Wiley & Sons, Inc.

GAREN, A. 1968. Sense and Nonsense in the genetic code. Science. *160* : 149.

GARROD, A. E. 1909. Inborn Errors of Metabolism. Oxford : Oxford University Press.

GOULIAN, M. 1971. Biosynthesis of DNA. Ann. Rev. Biochem. *40* : 855.

———, A. Kornberg and R. SINSHEIMER. 1967. Enzymatic synthesis of DNA : XXIV : Synthesis of infectious phage ϕX 174 DNA. Proc. Natl. Acad. Sci., U. S. *58* : 2321.

HADRON, E. 1968. Transdetermination in cells. Sci. Amer. *219* : 110.

HARRIS, H. 1970. Nucleus and Cytoplasm. 2nd ed. Oxford : Oxford University Press.

HAYES, H. K., F. R. IMMER and D. C. SMITH. 1955. Methods of Plant Breeding. 2nd Ed. New York : McGraw-Hill Book Company.

HAYES, W. 1968. The Genetics of Bacteria and their Viruses. New York : Wiley.

HECHT, M. K. and W. C. STEERE. 1970. Essays in Evolution and Genetics (in honour of Theodosius Dobzhansky). New York : Appleton-Century-Crofts.

HENDERSON, S. A. 1970. The time and place of meiotic crossing over. Ann. Rev. Genetics. *4* : 295.

HESS, O. and G. F. MEYER. 1968. Genetic activities of the Y-chromosome in *Drosophila* during spermatogenesis. Adv. Genetics. *14* : 171.

HOLLAENDER, A. 1971-73. Chemical mutations : Principles and Methods for their detection. Vols. 1, 2 and 3. New York : Plenum.

HOOK, E. B. 1973. Behavioral implications of the human XYY genotype. Science. *179* : 139.

HUBERMAN, J. A. 1973. Structure of chromosome fibres and chromosomes. Ann. Rev. Biochem. *42* : 355.

INGRAM, V. 1972. Biosynthesis of macromolecules. New York : Benjamin.

JACOB, F. and J. MONAD. 1961. On the regulation of gene activity. Cold Spring Harber Symp. Quant. Biol. *26* : 193.

JACOBS, P. A. and J. A. STRONG. 1959. A case of human intersexuality having a possible XXY sex determining mechanism. Nature. *183* : 302.

JENKINS, J. B. 1967. Genetics. Boston Houghton : Miffin.

JINKS, J. L. 1964. Extrachromosomal inheritance. Prentice-Hall, Englewood Cliffs, N. J.

JOHNS, E. W. and T. A. HOARE. 1970. Histones and gene control. Nature. *226* : 650.

KACHROO, P. 1975. Advancing Frontiers in Cytogenetics. (Prof. P. N. Mehra Commemorative Volume), Delhi : Hindustan Publishing Corporation.

KHORANA, H. G. 1966-67. Polynucleotide synthesis and the genetic code. Harvey Lectures *62* : 79.

KIHLMAN, B. A. 1971. Molecular mechanism of chromosome breakage and rejoining. Adv. Cell Mol. Biol. *1* : 59.

KLEIN, A. and F. BONHOEFFER. 1972. DNA replication. Ann. Rev. Biochem. *41* : 301.

LEDERBERG, E. M. 1956. Genetic transduction. Amer. Scientist *44* : 264.

LEVINE, L. 1969. Biology of gene. Saint Louis : The C. V. Mosby Co. and Tokyo : Toppon Company, Ltd.

MESELSON, M. 1964. On the mechanism of recombination between DNA molecules. J. Molec. Biol. *9* : 734.

——— and F. W. STAHL. 1958. The replication of DNA in *E. Coli.* Proc. Natl. Acad. Sci. U. S. *44* :671.

MITCHISON, J. M. 1971. The Biology of the Cell Cycle. Cambridge : Cambridge University Press.

MITTWOCH, U. 1973. Genetics and Sex Differentiation. New York : Academic Press.

MIZUTANI, S. and H. M. TAMIN, 1970. An RNA-dependent DNA polymerase in virions of Rous Sarcoma Virus. Cold Spring Harbor Symp. Quant. Biol. *35* : 847.

MORGAN, T. H. 1910. Sex-linked inheritance in *Drosophila*. Science *32* : *120*.

MOSIG, G. 1970. Recombination in bacteriophage T_4. Adv. Genetics. *15* : 1.

MULLER, H. J. 1916. The mechanism of crossing over. Amer. Nat. *50* : 193, 284, 350, 421.

NAYAR, N. M. 1973. Origin and Cytogenetics of rice. Adv. Genetics. *17* : 153.

NIRENBERG, M. and P. LEDER. 1964. RNA codewords and protein synthesis. Science. *145* : 1399.

NOTANI, N. K. and J. K. SETLOW. 1974. Mechanism of bacterial transformation and transfection. Prog. Nuc. Acid. Res. Molec. Biol. *14 : 39*.

PASTAN, I. 1972. Cyclic AMP. Sci. Amer. (Aug.).

PAUL, J. 1972. General theory of chromosome structure and gene activation in eukaryotes. Nature. *238* : 444.

PRESCOTT, D. M. 1970. The structure and replication of eukaryotic chromosomes. Adv. Cell. Biol. *1* : 57.

RAACKE, I. D. 1971. Molecular Biology of DNA and RNA : An Analysis of papers. St. Louis : Mosby.

RADDING, C. M. 1973. Molecular mechanism in genetic recombination. Ann. Rev. Genetics *7* : 87.

REZNIKOFF, W. S. 1972. The operon revisited. Ann. Rev. Genetics. *6* : 133.

RHOADES, M. M. 1946. Plastid Mutations. Cold Spring Harbor Symp. Quant. Biol. *11* : 202.

RIS, H. and D. F. KUBAI. 1970. Chromosome structure. Ann. Rev. Genetics. *4* : 263.

RIZVI, S. 1973. Colchiploidy in *Dimorphotheca aurantiaca.* Plant Science *5* : 39.

ROY, R. P. 1974. Sex mechanism in higher plants. J. Ind. Bot. Soc. *53* : 141.

SADGOPAL, A. 1968. The genetic code after the excitement. Adv. Genetics. *14* : 325.

SAGER, R. 1972. Cytoplasmic genes and organelles. New York : Academic Press.

SINGLETON, W. R. 1967. Elementary Genetics. New Delhi : Affiliated East-West Press Private Ltd.

SMITH, J. D. 1972. The genetics of *t*RNA. Ann. Rev. Genetics. *6* : 235.

SPIEGELMAN, S. 1964. Hybrid nucleic acids. Sci. Amer. (May).

SPIRIN, A. S. 1969. Inferosomes. Europe. J. Biochem. *10* : 20.

STEBBINS, G. L. 1966. Chromosomal variation and evolution. Science. *152* : 1463.

———. 1971. Processes of Organic Evolution. Englewood. Cliffs : Prentice-Hall.

STENT, G. S. 1963. Molecular Biology of Bacterial Viruses. San Francisco : Freeman.

———. 1965. Papers on Bacterial Viruses : Little, Brown.

———. 1971 Molecular Genetics : An Introductory Narrative. San Francisco : Freeman.

SUSMAN, M. 1970. General bacterial genetics. Ann. Rev. Genetics. *4* : 135.

SWANSON, C. P. 1957. Cytology and Cytogenetics. New York : Prentice Hall.

TAYLOR, A. L. 1965. Selected papers on Molecular Genetics. New York : Academic Press.

THOMAS, C. 1974. The rolling helix : a model for the eukaryote gene. Cold Spring Harbor Symp. Quant. Biol. *38* : 347.

THOMAS, C. A. 1971. The genetic organisation of chromosomes. Ann. Rev. Genetics. *5* : 237.

VOELLER, B. 1968. The chromosome theory of Inheritance. New York : Appleton-Century-Crofts.

WAGNER, R. P. and H. K. MITCHELL. 1964. Genetics and Metabolism. New York : Wiley.

WATSON, J. D. 1968. The Double Helix. New York : Atheneum.

————. 1970. Molecular Biology of gene. New York : Benjamin.

———— and F. H. C. CRICK. 1953 Molecular structure of nucleic acids : a structure for deoxyribose nucleic acid. Nature. *171* : 737.

WEINBERG, R. A. 1973. Nuclear RNA metabolism. Ann. Rev. Biochem. *52* : 329.

WESTERGAARD, W. and D. von WETTESTEIN. 1972. The synaptinemal complex. Ann. Rev. Genetics. *6* : 71.

WHITEHOUSE, H. L. K. 1969. Towards an Understanding of the Mechanism of Heredity. London, E. Arnold.

WILKIE, D. 1964. The Cytoplasm in Heredity. London : Metheu.

WILKINS, M. H. F. 1963. The molecular configuration of nucleic acids. Science *140* : 941.

WOESE, C. R. 1967. The Genetic Code : The Molecular Basis for Genetic Expression. New York. Harper and Row.

ZIMMERING, S., L. SANDLER and B. NICOLETTI. 1970. Mechanism of meiotic drive. Ann. Rev. Genetics. *4* : 409.

ZUBAY, G. and J. MARMUR. 1973. Papers in Biochemical Genetics. New York : Holt, Reinhart and Winston.

■ ■ ■

INDEX

abortive transduction 275
accessory-chromosomes 61
Ac-Ds position in *Zea mays* 260
Acetabularia 2, 4, 48
Acrocentric 53
Aegilops speltoides 197
A. squarrosa 197
Aging 42
Alcaptonuria 262
aleuroplasts 26
Alleles 122
Allelomorph 122
Allium sepa 53, 189
Allopolyploidy 187
allosomes 203
Amino acid activation 307
Amitosis 67
Amoeba 2, 23
amphiastral 70
amphidiploidy 187
amyloplasts 26
Anacystis nidulans 273
Anaphase 70, 81
Anemone 112
Aneuploidy 181, 182, 189
Anorthoploids 180, 181
Anteroposterior gynanders 212
Antirrhinum 143, 191
Arachis hypogea 362 364, 371
Archaeopteryx 344
arginine 254
Arisaema 111
Arisoemia 216
Artificial selection 361
Artificial synthesis of life 322
Artioploidy 181
ascus 247
Asparagus 339
Aulacantha 53
Australian region 343
Autoallopolyploids 189
Autolysis 42
Autophagy 42
Autopolyploidy 184
Autosomes 202, 209
autosyndesis 185
Autotetraploids 186
Autotriploids 185
Avena abyssinica 196
A. brevis 196
A. sativa 196

Bacillus subtilis 269
Back cross 123, 131
Back cross method 369
Bacterial transformations 269
Bagging 367
Balbiani ring 60
Barr body 208
bar eye 259
Base analogues—.
Basidiobolus 37
Batrachoseps 99
Beagle 351
Bees 213
Belling's hypothesis 100
Bellevalia 65
Benson's model 18
Bilateral gynanders 212
Biochemical genetics 246
Biochemical mutation 235, 253
Biogenetic law 334
Biomembrane 13
bioplast 33
bivalents 77
Bleeder disease 222
Bonellia viridis 206
Bonneville 243
bonquet 75
Bos taurus 279
B. P. Pal 397
Branton and Park's model 29
Brassica campestris 183, 364
B. juncea 188
B. napella 183
B. napus 188

B. oleracea 187
Breakage-fusion bridge 156
Breeding for disease resistance 373
Bryonia alba 212
B. dioica 212
Bulk method 369

Cairn's autoradiography 296
Cannabis sativa 214
Combodias 362
Capsicum annuum 186, 191
Carrica papaya 214
Carrier protein 14
Caffeine 237
Casts 344
Cell cycle 63
Cellula 1
Cell theory 1
Cell wall 10
Central dogma 306
Centriogene 166
Centrosome 44
Cercospora arachidicola 375
Chemical mutagens 237
Chemical evolution 327
Chiasmata 79
Chironomus 59
Chhoti lerma 245
Chlamydomonas 2, 4, 26, 45, 175
Chlorella 26
Chloroplasts 26
Choanichthyes 337
Cholesterol 14
Chondriokont 33
Chondriogene 166
Chondriome 33
Chondriosome 33
Chromoplasts 25
Chromosomal aberrations 156
Chromosomal map 102
Chromosomal theory of sex determination 202
Chromosomes 52
Cilia 45
Cistron 258
Cisternae 37
Citronellal 237
Citruline 254
Cladophora 49
Classical hypothesis 99
Classification of mutation 234
ClB method 231
Clematis 112
Clintonia 113
Clone and clonal selection 364
Coacervate drops 331
Coccinia 214
Codon's dictionary 318
Colchicine 191, 237
Colchicine automnale 191
Colour blindness 220
Complementary factors 145
Complete transduction 275
Complon 258
Composite behaviour of ribosomes 309
Composite cross method 370
Composite model 19
Compressions 343
Conservative method 292
Constitutive mutants 325
Corchorus capsularis 364
CO sensitivity 175
Cotton 396
Coumarine 197
Coupling and repulsion phenomenon 90
Crepis foetida-rubra 189
Criss cross inheritance 219
Cristae 34
Crop breeding in India 386
Crossing 368
Crossing over 97
Crossing over mechanism 97
Croton 167
Cucumis 216
Cucurbita 155, 191 ,214
Cumulative factors 152
Cuscuta 339
Cylindrospermum majus 273
Cyperales 114
Cytokinesis 67, 72
Cytoplasmic genetic system 165
Cytoplasmic heredity 165, 166
Cytosterility 176
Cytotaxonomy 110

Danielli-Davson hypothesis 15
Darwin 351
Darwinism 351

Datura stramonium 183, 189, 190
D. ferox 183
Davenport's experiment 354
Deficiencies 156
Dee-gee-woo-gen 243
Delphinium 189
deoxyadenylic acid 282
deoxycytidilic acid 282
deoxyguanylic acid 282
deoxythymidilic acid 282
Desmosomes 21
Detection of biochemical mutation 252
Detection of mutation 231
Detection of mutation in plants 233
deuteranopia 221
Dharwar American 362
Diakinesis 80
Didelphis 80
Differences DNA and RNA 299
Dihybrid cross 122, 128
Dimorphotheca aurantiaca 186
Diplococcus pneumoniae 269
Diosporum 113
Diprion 194
Diplotene 78
Dispersive method 292
DNA 279
DNA model 284
Dodahatti 362
Dominant 122
Dominant factor hypothesis 381
double bar eye 259
Dread disease 222
Drosophila 21, 160, 213 etc.
drum-sticks 209
Duplicate factors 149
Duplications 157
dwarf wheats 243

elaioplasts 26
Eleocharis 5
Emasculation 367
Eohippus 344
epigamic 202
Epilobium 172
Epistasis 147
Equisetum 216
Equus 345
Escherichia coli 8, 14, 41, 230, 238 etc.
Evolution of horse 344
Euchaena 112
Eudorina 4
Euglena 4, 28,33, 167
Euploidy 181, 182
Evidences from anatomy 339
,, ,, classification 342
,, ,, comparative 338
,, ,, domestication 341
,, ,, embryology 334
,, ,, fossils 343
,, ,, genetics 341
,, ,, geographical distribution 342
,, ,, physiology 340
,, ,, vestigeal organs 339
Evidences of evolution 334-343
Ethiopian region 343
Extra-nuclear inheritance 165
Eye pigments in *Drosophila* 264

Feed back inhibition 322
Filial generation 122
Flagella 45
Floral pigmentation 262
Fluid mosaic model 19
Folded-clover leaf model 303
Folded fibre model 58
forbidden base pairs 241
Forked-line method 136
Formation of polypeptide chain 313
Fragaria 189
Fusarium oxysporum 37

Gamma garden 237
Gamma rays 237
Gene 255
Genes and chromosomes 255
Genes at the core of crime 261
Genes, definition 255, 256
Genes, nature 256
Genetic code 315
Genetics of *Neurospora* 247-254
Genic balance theory 204
Genomic alloploids 188
Genotype 122
Germplasm 357
Germinal mutation 235
G_1, G_2 phases 63

Ginkgo 343
Giraffe 349
Gloriosa superba 188
glycolate metabolism 44
glycolipids 14
Glyoxysomes 43
Golgi bodies 39
Golgi complex 39
Gossypium 96, 199, 364
G. anomalum 192, 199
G. arboreum 196, 199, 371, 372
G. barbadense 183
G. davidsonii 183, 199
G. herbaceum 199, 371, 372
G. hirsutum 183, 196, 371,
Grana 27
Greater initial capital theory 380

Hammerling's experiment 48
Haploids 182
Haplopappus 53
Helianthus tuberosus 189
hemicellulose 11
Hemophilia 222
Hemophilous 269
hereditary blood disease 265
Heteroploidy 182, 189
Heterosis 374
heterotypic division 74
Heterozygosity hypothesis 380
Heterozygous 122
hexuronic acid 11
Hipparion 346
Hirudinea 194
HMC 290
homogentisic acid 262
homotypic division 74
Homozygous 122
Hordeum vulgare 183
Hormonal control of Sex determination 206.
Hybrid vigour 374
Hydrilla 5
Hydrodictyon 49
Hydroxylamine 238
Hydrophobic binding model 17
Hypohippus 346
Hyperploidy 181
Hypoploidy 181

IAA 254
Incomplete dominance 142
Induced mutations 236
Induction of polyploidy 191
Infective RNA 276
Inheritance of acquired character 348
Inheritance of coiling in *Lemnaea* 178
Inheritance test in *Neurospora* 249
Inhibitory factors 148
IR 8
Interaction between dominant genes 143
Intergeneric hybridisation 366
Interkinesis 82
Interspecific hybridisation 365
Intervarietal hybridisation 365
Intravarietal hybridisation 365
Introgressive hybridisation 366
Inversion 161
iojap 168
isoleucine synthesis 322

Janssen's hypothesis 99
japonica 244

Kalyan sona 245
Konamani 243
Karyokinesis 67
Kavanau's lipid pillars 16
Killer character 173
kinetigene 166
Klinefelter's syndrome 209
Kreb's cycle 36

Labelling 368
Lactose operon model 323
Lactose repressible system 323
Lamarck 347
Lamarckism 347
Lampbrush chromosomes 60
Lathyrus odoratus 95
Law of dominance 123
Law of independent assortment 128
Law of segregation 125
Lemnaea peregra 178
Lenard and Singer's model 18
Leptotene 75
Lerma Rojo 245
lethal factors 151

leucoplasts 25
leutin 31
linkage 90
linkage in *Drosophila* 90
linkage in *Zea mays* 92
linkage theories 95
linked genes 90
lipochondria 39
lotus 189
Lycopersicum 183, 371 etc.
Lysosomes 41

M 63
Mass selection 361
mauve 263
Mc 290
Mechanism of mutation 235
Medicago hispida 196
M. sativa 196
Meiosis 74
Melandrium 209, 215
Membrane DNA 15
Membrane protein 13
Membrane RNA 15
Mendelism 115
Mentha piperita 243
Mercurialis 216
Merychippus 345
Meselson & Stahl's expt. 294
Mesohippus 345
Messenger RNA 300
Mesostoma 194
Metabolic theory of sex determination 204
Metacentric 53
Metaphase 70
Methionine synthesis 254
Methods of crop improvement 360
micelle 11
Microbial genetics 269
microbodies 34
Microfibrils 12
Microspheres 331
Microtubules 43
Microvilli 20
Middle lamella 11
Miller's experiment 328
Miohippus 346
Mirabilis 142, 165, 170 etc
Misincorporation 239
Misreplication 240
Mitochondria 33
Mitosis 67
Mnium 27
Modifications to Watson & Crick model 288
Monohybrid cross 125
Monosomic aneuploidy 190
Monosomics 181
Morus alba 214
Mosaic membrane concept 18
moulds 344
Mouse milk factor 179
M. S. Swaminathan 398
mule 374
Muller's 5 method 233
Multiple factors 152
Mutations 216
Mutation theory of evolution 357
Muton 258
Mycoplasma 13

N-acetylglucosamine 14
Nasse's law 220
Natural selection 353, 361
Nature of genes 256
Nature of genetic code 318
Nearctic region 343
Neo-Darwinism 356
Neodiprion 194
Neotropical regions 343
Nepeta 169
Neurospora 37, 175, 247-254, etc.
Nicotiana 155 183, 188, 189, 199, 371 etc.
Nitella 5
Nitrous acid 238
non-disjunctional division 81
Non-disjunction in *Drosophila* 226
Norin gene 243
Nuclear ribosomes 51
Nucleolus 51
Nuclear membrane 50
Nucleoside 281
Nucleus 48
Nullisomics 181, 190
Nutritional theory of Sex-determination 204

Nymphaea 25

Oedogonium 26
Oenothera 160, 167, 169, 183, 230, 357 etc.
Oligosomy 181
One gene-one enzyme hypothesis 262
One gene-one polypeptide hypothesis 267
Ontogeny of aortic arches 336
Ontogeny of hearts 336
Operator constitutive 325
Operator gene 323
Operon 259
Operon model 323
Origin of biological macromolecules 330
Origin of first living system 330
Origin of gynanders 212
Origin of life 326
Origin of new species 354
Ornithine 254
Orthoploids 180
Orthoptera 255
Oryza sativa 96, 183, 364, 371, 372 etc.
osmophilic granules 27
Oxysomes 34
Overproduction 342
Pachytene 78
Paeonia 160, 189
Paleartic region 343
Palmales 114
Pandorina 4
Panicum 145
paracentric inversion 163
Paramoecium 2, 167, 172
paranemic coiling 69
Pearl millets 385
Pedgree method 369
Pennisetum 215, 362
pericentric inversion 163
Perissoploidy 181
Peroxisome 44
Petite colonies 174
petrifactions 344
Petunia 191
Phagein 22
Phagocytosis 22
Phaseolus vulgaris 35
phenotype 122
Philosophic Zoologique 347
Phleum pratense 189
photorespiration 44
Photosynthetic units 31
phycobilisomes 26
Physiology of cell division 85
Pinocytosis 23
Pithophora 49
Plantago 111
Plant breeding 360
plasmagene 166
plasmodesmata 4
Plasmon 166
Plastid inheritance 167
Plastids 25
plastogene 166
plastoquinone 31
plectonemic coiling 69
Pleiotropy 266
Pliohippus 345
Poky characters 175
Poliomyeletis 276
Polygonales 114
Polygonatum 113
Polyhybrid cross 133
Polymorphism 150
Polyploidy 180
Polyploidy in animals 192
Polyploidy in evolution 194
Portulaca grandiflora 183, 191
Position effect 259
Position effect in *Drosophila* 259
Position effect in *Zea mays* 260
Potato 365, 391
Poulton & Sander's expt. 354
Pre-biotic chemical evolution 328
Primary non-disjunction 227
Primary wall 11
Primula 111
Procaryotic cells 7
Procedure of hybridisation 366
Progamic 202
promoter gene 323
Prophase I, 74
protanopia 221
protoperithecium 248
Protoplasm 4
Protoplasm theory 1

Protoplast 4
Pyrus malus 186

Quantasome 31
Quantitative inheritance 152
Quantitative theory of sex determination 204

Radiations 236
radio active clock 344
Raphanobrassica 187, 191, 341
Raphanus sativum 187
Rate of replication 297
recapitulation theory 334
Recessive 122
Recon 258
Red Triumph 243
Regulation of gene activity 321
regulator constitutive 325
regulator gene 323
Replication of DNA 291
Replicon 258
Repressible system 323
repressor 323
Reverse mutation 235
Rhizopus 2
Ribosomal RNA 300
Rice 389
Riccinus 216
RNA 298
Role of *y* chromosome 207
Rous cercoma virus 278
Royal disease 222
Rubus 188
Rumex 212
Ruscus 339
Russet Burbank 243

S 63
Saccharum 372 etc.
Salamander maculosa 349
Salivary gland chromosome 59
Salmon 263
Salmonella typhimarium 14, 274
Secale 188, 197
Secondary non-disjunction 227
Secondary trisomics 190
Secondary wall 11
Segmental allopolyploids 188
Selection 361
Semiconservative method 292
Sex determination 201
Sex determination in *Drosophila* 206
Sex determination in higher plants 210
Sex determination in man 208
Sex index ratio 205
Sex linked inheritance 218
Sex linked inheritance in *Drosophila* 218
,, ,, ,, man 220
,, ,, ,, poultry 224
Sex linked lethals in *Drosophila* 226
Sex piebalds 212
Sex reversal 201, 214
Sharbati Sonara 245, 246
Sialic acid 14
Sickle-cell anaemia 265
Silk moth 213
Single stranded DNA 289
Size of gene 257
Smilacina 113
Smooth Burbank 243
Snapdragon 143
Solanum tuberosum 183, 188, 362, 371, 372 etc.
Somatic mutation 235
Somatoplasm 357
Some unusual bases of DNA 290
Sonalika 245
Sonara *64* 245
Sorghum 96, 146, 362 etc.
S. *halepense* 188, 196
S. *vulgare* 196
Spherosomes 44
Spinach 44
Spirogyra 26, 45, 70
Spontaneous mutation 235
Spurious mutation 235
s RNA 307
Stability of gene 259
status albomaculatus 168
Stern's experiment 106
Streptocarpus 262
Streptomycin resistance 175
Stroma 27
stromatin 21
structural gene 323
Struggle for existence 352

Submetacentric 53
Sugar cane 394
Suicidal bags 42
Superdominance hypothesis 381
Sun flower 44
Supernumery chromosome 61
Supplementary factors 146
Suppression 266
Survival of fittest 353
Symplast 4
synapsis 77
syngamic 202
syninzesis 75
Synthetic theory of evolution 359
Taichung *65* 244
Taichung-Native*1* 244
Taiwan*3* 244
Talaivirichan 243
tautomeric shifts 241
Telocentric 53
Telophase I, 81
Test cross 123, 131
tetrads 77
Tetrasomics 181
Theory of germ plasm 356
Theories on evolution 347
thylakoid 29
thymine dimers 236
Torsion theory 100
Tradescantia 5, 64, 191
Transcription 258, 306
Transduction 274
Transfer RNA 302
Transformation 269
transition 240
Translation 307
Translocation 159
Transosomes 43
transversion 240
Treptophan-less mutants of
  Neurospora 264
Trichoniscus 186
Trifolium hybridum 196
T. medium 196
T. repens 196
Trihybrid cross 133
Trillium 53, 65
Tripsacum 31
Trisomics 181
Triticale 187, 188, 197, 341 etc.
Triticum 111
T. aegilopoides 197
T. aestivum 196, 364, 371, 372
T. compactum 183, 196
T. dicoccum 183 196, 371
T. durum 371
T. monococcum 183, 196
T. persicum 183
T. spelta 199
T. vulgare 183, 189, 196
Triumph 243
Tryptophan 254
Turner's syndrome 209

Ultrastructure of chloroplast 29
Ultra violet light 236
Unit membrane hypothesis 15
Unusual bases in DNA 290
Unusual bases in RNA 303

Vacuole 6
Vaucheria 2
Viccia faba 64, 65

Watson & Crick model 284
,, ,, ,, modification 288
Weier and Benson model 30
Weldon's experiment 354
Wheat 386

X-rays 236

Yeast 2, 35, 174

Zea mays 168, 183, 189, 211, 362,
  384 etc.
Zygotene 76
Zygnema 26

■ ■ ■

S.C.E.R.T. West Bengal
Library
Calcutta
28.3.

[illegible]

Cytogenetics, Evolution & Plant Breeding
T 755/79—60153016 Rs. 18.00